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182. VARIATIONS IN THE PROTEIN CONTENT OF MILK DURING LACTATION

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(With 12 Figures)

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INTRODUCTION

THE object of this investigation was to study the genetical aspect of the protein, and particularly the casein, content of the milk of dairy cattle. Unless the whole lactation period be studied, it is not possible to obtain an accurate figure for the lactation yield. Since genetical research demands the analysis of the yields of a large number of animals, and since the method of analysing the content of the milk in respect of protein and casein is complicated and time consuming, it was obvious, at the outset, that detailed information was required on the variation of the content of the milk in these respects according to the stage of lactation. In other words, an answer was required to the question: "How frequently during the lactation period is it necessary to measure the protein and casein content of the milk of a cow in order to obtain a reasonably accurate figure for the lactation yield of that cow?" To answer this question detailed information was required concerning the week to week variation in the lactation period. Two possible methods of doing this appeared to be:

(1) To analyse regularly at short intervals the milk of several cows from the beginning until the end of their lactations and from the figures obtained to deduce the general trend and the extent of these variations for the whole lactation time.

(2) To take a greater number of cows at different stages of lactation and analyse their milk at short intervals *during a few months only*. Several cows being under examination at the same time for any stage of lactation, the

analysis of the figures obtained would enable us to explore step by step the whole lactation curve with regard to the variations in question.

The second method curtailed the time required for the whole experiment and was adopted.

Material

Twenty-seven cows were selected from the experimental farms of the Institute, where the animals are kept under standard conditions. The system of rationing is kept constant and the quantity of the feeding depends only upon the yields of the cows. Differences in the composition of the milk may therefore be attributed to age, stage of lactation, and the individuality of the cow.

The particulars of these cows, which may be of interest here, are set out in Table I.

Table I. *Resuming the material of work*

Cow no.	Breed	Age in months	Period of lactation in which the cow was under examination
1	Beef/Dairy Shorthorn	60	1st–11th week
2	Beef Shorthorn	64	1st–12th ,,
3	Ayrshire	87	1st–11th ,,
4	Ayrshire	79	1st–14th ,,
5	Shorthorn/Ayrshire	53	3rd–13th ,,
6	Beef Shorthorn	37	3rd–19th ,,
7	Jersey	44	5th–21st ,,
8	Jersey	50	5th–21st ,,
9	Beef/Dairy Shorthorn	60	6th–22nd ,,
10	Shorthorn/Ayrshire	59	8th–18th ,,
11	Ayrshire	67	11th–27th ,,
12	Dairy/Shorthorn	68	15th–31st ,,
13	Dairy/Beef Shorthorn	48	15th–31st ,,
14	Ayrshire	43	20th–36th ,,
15	Dairy/Beef Shorthorn	52	20th–36th ,,
16	Ayrshire	36	21st–39th ,, (dried off)
17	Ayrshire	41	21st–37th ,,
18	Ayrshire	74	22nd–38th ,,
19	Ayrshire	44	22nd–38th ,,
20	Ayrshire	42	23rd–39th ,,
21	Ayrshire	40	24th–40th ,,
22	Ayrshire	75	25th–41st ,,
23	Jersey	63	31st–42nd ,, (dried off)
24	Beef Shorthorn	88	40th–41st ,,
25	Ayrshire	100	40th–52nd ,,
26	Ayrshire	74	40th–51st ,, (dried off)
27	Ayrshire	75	44th–46th ,, (dried off)

Within the periods given in this table the milk of each cow was regularly analysed at intervals of 7 days. Occasionally, due to unavoidable circumstances, this interval was 8 or 9 days. The work was carried on for the majority of the cows during 18 weeks. This period was shorter for some of them either because they dried off before the end of the work, or they had not yet calved when it was started, etc.

Methods

Sampling. All the usual precautions were taken in the collection of the samples.

The cows on the experimental farms are usually milked twice daily at an

interval of 12 hr. Variation in the interval rarely amounts to more than 15 min. A few of the high-yielding cows are, for a portion of their lactation, milked three times a day at 8 hr. intervals. The amount of milk collected from each cow was weighed to the nearest quarter of a pound, and samples taken from each milking of each cow were placed in a refrigerator and sent on the following morning to the Institute. In the laboratory representative samples of each cow's milk for 24 hr. were then obtained by mixing appropriate quantities of milk from each bottle.

Chemical analysis. The total protein nitrogen was determined by precipitation by trichloracetic acid and a Kjeldahl digestion, as described by Sanders (1).

In order to test the reliability of the methods of determining casein, results obtained by the volumetric (Van Slyke (2)), colorimetric (Leroy (3)) and official methods were compared. The first two, though valuable for practical purposes and cheese-making on account of their rapidity, did not seem to be accurate enough for this investigation. The official method was therefore adopted with the improvement recently introduced by Moir (4), where the casein is precipitated at its isoelectric point by the use of a buffer solution of acetic acid and sodium acetate.

From the well-mixed sample prepared as above, 10 ml. were pipetted into a tared weighing bottle and weighed to the nearest centigram. Two other 10 ml. portions were pipetted into covered beakers. The weight of the milk in the weighing bottle was taken to be the same as that of the milk pipetted into the beakers. This is generally true, but slight differences may be observed when the sample is very creamy and particles of the cream stick against the side of the pipette. This point has been taken into consideration when determining the standard error.

Determination of total protein nitrogen. Ten ml. of a 2·43 N (approximately 10%) trichloracetic acid solution were added to one of the beakers mentioned above; the protein was completely precipitated at once, and filtered through a 12·5 cm. No. 12 Whatman filter-paper. The Biuret test was tried in the filtrate and negative results were always obtained. In order to remove the part of the precipitate adhering to the side of the beaker about 20 ml. of distilled water were added to the beaker and 5 or 6 ml. of nitrogen-free concentrated sulphuric acid were poured down the side of the beaker; the remainder of the precipitate dissolved by the heat produced in this mixture was poured into a Kjeldahl flask. This was repeated several times and completed each time by scraping a glass rod against the side of the beaker until the removal was complete. Finally, the filter paper and the precipitate were added to the Kjeldahl flask. The digestion, distillation, and titration were carried out in the usual way, selenium (about 0·08 g.) being used as catalyst during the digestion.

Determination of casein nitrogen. The milk placed in the second beaker was diluted with about 50 ml. of distilled water at 40–42° C. One and a half ml. of a 1·67 N acetic acid solution were added immediately after the water, and the mixture was gently stirred. The beaker was then left to stand for about

5–10 min. after which 4·5 ml. of a 0·25*N* sodium acetate solution were added, and the mixture was allowed to stand for 30–40 min. before being filtered. Moir recommends an interval of 20 min. between the additions of acetic acid and sodium acetate, and a further interval of 1 hr. before filtration. These intervals, as McDowall & McDowell have pointed out, "seem unnecessarily long, as neither the final *p*H nor the casein result is affected by adopting much shorter intervals, e.g. 5 and 15 min. respectively" (5). After the precipitate had settled, it was washed three times by decantation and transferred to the filter-paper by two further washings. The rest of the experiment was carried out as for the total protein.

Determination of albumin + globulin nitrogen. This was done for each sample by subtracting the casein nitrogen from the total protein nitrogen.

Blank experiments were carried out from time to time and the proper correction applied to the results.

Errors. None of these determinations was made in duplicate. The systematic errors were reduced to a minimum by always using the same pipette, starting each measurement with burettes at the same graduation mark, using the same quantities of indicator, and so on. But even so, no reliable information could be drawn from the figures obtained, if we had not an exact idea of the magnitude of these errors. This was determined by analyses carried out on a sample of milk, from cow No. 8, a Jersey cow whose milk was generally very creamy and left particles on the side of the pipette. Ten determinations of the total protein nitrogen, and ten determinations of the casein nitrogen were carried out on this sample; the albumin + globulin nitrogen was calculated each time by difference. The mean and the standard error of the results obtained are set out below for each case:

	Mean of ten determinations made on the same sample	Standard error
Total protein nitrogen	0·5921	±0·0043
Casein nitrogen	0·4788	±0·0047
Albumin + globulin nitrogen	0·1133	±0·0066

Thus the standard error for casein nitrogen is higher than for total protein nitrogen. In the case of albumin + globulin nitrogen the standard error was also determined theoretically by using the formulae

$$\sigma_{A.+G.} = \sqrt{(\sigma_p^2 + \sigma_c^2)},$$

and the result ±0·0064 was close to the figure given above.

For a less creamy sample which would leave the pipette clearer these errors would probably be smaller.

Analysis and interpretation of the data

The statistical analysis of the figures was made by "methode des couples" (6) or the method of "pairing". This method has been used in many fields of biological investigations, but as far as the writer knows it has not been used for the study of variations of the kind under examination. Its application to

these data was considered not only legitimate, but as being the best means of securing reliable information from such a small number of observations.

All it was hoped to get from these data was the answer to the question: Does the protein content of milk vary during the lactation period, and if so, how? A positive answer to the first part of the question was easily obtained from the examination of the figures of individual cows, as the variations observed in the milk of some of them were sometimes far beyond the limits $\pm 2\sigma$ which could be ascribed to the systematic errors of the experiment. To get the answer to the second part of the question was somewhat more difficult and much more laborious. The differences observed in the composition of the milk of the cows under examination could be ascribed, as mentioned above, to the interaction of the breed, age, stage of lactation, heredity, individuality of the cow, and probably to some other unknown causes. By the use of the method known as "factorial arrangement" and the analysis of the arrangement so obtained, it is possible, though not always easy, to deduce the way in which each single factor interacts with the others; in other words to find what would be the influence of one definite factor if all the others were eliminated. Hence to study the influence of the lactation factor, with which this paper is concerned, we classified our data according to the weeks of lactation as shown in Tables II, III and IV; and the method of "pairing" was applied to this arrangement.

Owing to the fact that the length of lactations is different for different cows, and that only data for parts of lactation were available, the "factorial arrangement" was carried out separately for the first 30 and the last 16 weeks of lactation.

In each of Tables II, III and IV the figures corresponding to any week of lactation (vertical columns) were compared with those of the week immediately preceding as follows:

A "couple" or a "pair of figures" being defined here as two figures obtained from the milk of the *same cow* in *two* consecutive weeks, every figure in one column which could not be paired with a figure of the adjoining column was discarded from the comparison. Then each figure of the column $N+1$ was subtracted from the corresponding figure of the column N and the following statistics were determined:

The mean of the differences obtained,

The variance of these differences,

The variance of the mean of these differences,

The standard error of the mean of these differences, and the value:

$$t = \frac{\text{mean}}{\text{standard error of the mean}}$$

which was used to test the significance of the result by "Student's" table(7).

The two examples given below illustrate the details of the method.

Table II. *Milligrams of total protein nitrogen in* 100 *g. of milk*

Cow no.	Weeks in lactation														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	612	486	427	.	421	427	459	436	464	471	463	.	.	.	.
2	592	512	437	433	409	405	422	442	482	453	470	457	.	.	.
3	672	563	480	465	440	426	435	468	460	523	510	.	.	.	.
4	580	476	453	440	420	430	408	440	464	460	462	451	462	.	.
5	.	.	455	425	442	420	450	404	433	503	451	464	452	.	.
6	.	.	475	445	488	476	471	477	482	480	461	468	482	491	509
7	.	.	.	.	487	493	484	478	457	460	469	462	469	455	484
8	.	.	.	.	549	517	489	531	.	546	527	572	530	481	511
9	.	.	.	.	.	425	431	432	448	448	447	441	452	467	463
10	.	.	.	.	.	.	.	496	452	424	436	448	449	453	509
11	.	.	.	.	.	.	.	.	.	.	535	415	417	450	431
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	470
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	493

	Weeks in lactation														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6	528	514	527	505	.	.	.	.	.	.	.	.	.	.	.
7	505	498	552	550	555	570	.	.	.	.	.	.	.	.	.
8	484	496	519	521	543	539	.	.	.	.	.	.	.	.	.
9	442	452	476	.	503	509	504	.	.	.	.	.	.	.	.
10	460	492	494	.	.	.	.	.	.	.	.	.	.	.	.
11	452	446	430	445	440	418	449	445	489	467	468	481	.	.	.
12	484	508	526	499	514	532	510	486	508	502	492	537	547	525	535
13	.	477	500	492	.	513	472	493	482	457	460	513	504	500	536
14	.	.	.	.	556	679	585	607	589	608	595	590	582	516	545
15	.	.	.	.	503	498	465	492	480	466	500	468	495	496	489
16	.	.	.	.	.	483	540	547	498	504	496	489	480	473	500
17	.	.	.	.	.	516	523	540	537	514	508	524	505	509	469
18	.	.	.	.	.	.	462	443	571	448	460	459	475	443	438
19	.	.	.	.	.	.	651	545	558	545	555	555	542	548	543
20	.	.	.	.	.	.	.	525	513	517	554	538	534	527	522
21	.	.	.	.	.	.	.	.	487	.	552	484	518	511	495
22	.	.	.	.	.	.	.	.	.	573	480	551	608	572	644

	Weeks before the end of lactation															
	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
2	409	405	422	442	482	453	470	457	.	.	.	.	.	.	.	.
1	.	421	427	459	436	464	471	463	.	.	.	.	.	.	.	.
10	436	448	449	453	509	460	492	494	.	.	.	.	.	.	.	.
12	532	510	486	508	502	492	537	547	525	535	542	.	.	.	.	.
9	441	452	467	463	442	452	476	.	503	509	504	.	.	.	.	.
17	537	514	508	524	505	509	469	500	507	516	509	531	.	528	.	.
24	.	.	526	562	540	483	507	496	506	500	526	551	.	537	499	.
13	493	.	477	500	492	.	513	472	493	482	457	460	513	504	500	536
25	.	.	.	.	.	457	486	480	467	515	530	541	584	571	573	561
23	.	.	.	.	627	628	626	540	644	601	630	647	637	654	652	655
26	.	.	.	.	.	.	.	.	.	.	.	.	.	644	614	661
27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	511	634

Table III. *Milligrams of casein nitrgoen in* 100 *g. of milk*

Cow no.	Weeks in lactation														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	452	392	346	319	324	338	367	345	374	362	351	.	.	.	.
2	469	409	354	341	309	318	336	352	388	359	365	341	.	.	.
3	569	474	394	384	368	373	365	403	384	431	429	.	.	.	.
4	486	388	386	370	362	.	337	.	383	403	404	380	394	.	.
5	.	.	391	357	372	359	373	334	369	400	.	380	368	.	.
6	.	.	400	394	388	385	398	393	399	404	397	390	403	405	427
7	.	.	.	.	391	409	410	386	354	381	318	388	382	347	392
8	.	.	.	.	406	416	416	425	427	445	428	435	441	396	471
9	.	.	.	.	.	343	336	340	348	353	346	336	343	345	359
10	.	.	.	.	.	.	.	366	364	346	336	356	361	358	414
11	.	.	.	.	.	.	.	.	.	.	429	344	353	351	356
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	393
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	412

	Weeks in lactation														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6	440	418	431	.	.	.	.	.	.	.	.	.	.	.	.
7	386	407	458	447	456	467	.	.	.	.	.	.	.	.	.
8	430	409	420	440	437	446	.	.	.	.	.	.	.	.	.
9	321	341	370	.	375	387	354	.	.	.	.	.	.	.	.
10	358	411	406	.	.	.	.	.	.	.	.	.	.	.	.
11	377	356	367	355	350	325	353	348	464	.	372	367	.	.	.
12	404	418	426	413	.	443	414	405	412	403	390	442	454	430	.
13	415	359	422	406	413	426	383	409	399	385	394	416	409	409	417
14	.	.	.	.	488	473	483	481	488	505	493	477	.	478	451
15	.	.	.	.	393	399	402	400	378	385	392	372	392	392	356
16	.	.	.	.	.	399	395	344	407	413	388	404	402	380	409
17	.	.	.	.	.	419	423	470	442	416	424	439	381	418	380
18	.	.	.	.	.	.	396	362	382	376	403	394	369	366	360
19	.	.	.	.	.	.	573	418	434	424	424	426	425	430	415
20	.	.	.	.	.	.	.	440	416	432	441	431	438	436	423
21	.	.	.	.	.	.	.	.	418	.	461	430	446	436	434
22	.	.	.	.	.	.	.	.	.	501	474	479	528	444	524

	Weeks before the end of lactation															
	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
2	309	318	336	352	388	359	365	341	.	.	.	.	.	.	.	.
1	319	324	338	367	345	374	362	351	.	.	.	.	.	.	.	.
10	336	356	361	358	414	358	441	406	.	.	.	.	.	.	.	.
12	443	414	405	412	403	390	442	454	430	.	.	.	.	.	.	.
9	336	343	345	359	321	341	370	.	375	387	354	.	.	.	.	.
17	442	416	424	439	381	418	380	394	411	398	387	.	406	418	.	.
24	.	.	404	448	427	405	418	388	398	389	414	423	.	387	357	.
13	412	415	359	422	406	413	426	383	409	399	385	394	416	409	409	417
25	.	.	.	.	.	334	381	362	340	378	394	405	402	391	407	403
23	.	.	.	.	524	516	524	481	507	501	530	538	519	530	537	548
26	.	.	.	.	.	.	.	.	.	.	.	.	.	517	519	542
27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	356	458

Table IV. *Milligrams of albumin and globulin nitrogen in* 100 *g. of milk*

Cow no.	Weeks in lactation														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	160	94	81	.	97	89	92	91	89	108	111	.	.	.	.
2	123	102	83	92	100	87	86	90	94	93	105	117	.	.	.
3	102	90	86	81	72	54	70	65	76	91	81	.	.	.	.
4	94	87	68	70	58	.	71	.	81	57	58	71	68	.	.
5	.	.	63	68	70	61	76	70	64	103	.	84	85	.	.
6	.	.	75	51	100	91	73	84	82	76	64	78	80	86	82
7	.	.	.	.	97	84	74	92	102	79	150	74	88	108	92
8	.	.	.	.	144	101	73	105	.	101	99	92	89	84	40
9	.	.	.	.	.	81	95	92	101	95	101	105	108	122	104
10	.	.	.	.	.	.	.	130	87	77	101	91	88	95	94
11	.	.	.	.	.	.	.	.	.	.	106	71	63	99	75
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	76
13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	81

	Weeks in lactation														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6	88	96	96	.	.	.	.	.	.	.	.	.	.	.	.
7	119	91	94	103	99	104	.	.	.	.	.	.	.	.	.
8	55	88	99	81	106	93	102	.	.	.	.	.	.	.	.
9	120	110	106	.	128	122	150	.	.	.	.	.	.	.	.
10	102	82	88	.	.	.	.	.	.	.	.	.	.	.	.
11	75	90	63	90	90	93	96	97	25	.	96	113	.	.	.
12	81	90	100	86	.	89	96	82	96	99	102	95	93	95	.
13	.	118	78	86	.	86	90	84	84	72	66	97	96	91	119
14	.	.	.	.	68	207	102	126	101	103	102	113	.	38	94
15	.	.	.	.	110	99	62	92	102	81	108	95	103	104	133
16	.	.	.	.	.	84	145	203	90	91	108	85	78	93	91
17	.	.	.	.	.	97	100	70	95	98	84	85	123	92	89
18	.	.	.	.	.	.	67	81	189	72	56	65	105	77	78
19	.	.	.	.	.	.	77	127	124	121	131	129	117	118	128
20	.	.	.	.	.	.	.	85	97	85	113	107	96	91	100
21	.	.	.	.	.	.	.	.	68	.	91	54	72	75	61
22	.	.	.	.	.	.	.	.	.	72	5	72	80	128	120

	Weeks before the end of lactation															
	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
2	100	87	86	90	94	93	105	117	.	.	.	.	.	.	.	.
1	.	97	89	92	91	89	108	111	.	.	.	.	.	.	.	.
10	101	91	88	95	94	102	82	88	.	.	.	.	.	.	.	.
12	89	96	82	96	99	102	95	93	95	.	.	.	.	.	.	.
9	105	108	122	104	120	110	106	.	128	122	150	.	.	.	.	.
17	95	98	84	85	123	92	89	106	96	117	121	.	.	110	.	.
24	.	.	122	114	114	79	88	108	108	111	112	128	.	149	141	.
13	81	.	118	78	86	.	86	90	84	84	72	66	97	96	91	119
25	.	.	.	.	.	122	105	118	127	137	136	135	182	181	166	158
23	.	.	.	.	103	112	102	60	136	100	100	109	117	124	115	107
26	.	.	.	.	.	.	.	.	.	.	.	.	.	126	96	119
27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	155	176

Example 1. Comparison of the tenth with the eleventh week of lactation regarding the total protein content of milk (see Table II).

Cow no.	10th week	11th week	d	d^2
1	471	463	+ 8	64
2	453	470	−17	289
3	523	510	+13	169
4	460	462	− 2	4
5	503	451	+52	2704
6	480	461	+19	361
7	460	469	− 9	81
8	546	527	+19	361
9	448	447	+ 1	1
10	424	436	−12	144
11	—	535 (discarded)	—	—
			+112	
Totals	4768	4696	−40	4178
			+72	
Means	476·8	469·6	+7·2	417·8

Variance of the differences $= md^2 - (md)^2 = 417{\cdot}84 - 51{\cdot}8 = 365{\cdot}96$. Variance of the mean of the differences $\frac{365{\cdot}96}{9} = 40{\cdot}66$. Standard error of the mean of the differences $= \sqrt{(40{\cdot}66)} = 6{\cdot}376$. $t = \frac{7{\cdot}2}{6{\cdot}376} = 1{\cdot}13$. In consulting "Student's" table we will see that for $n = 9$ (n is the number of degrees of freedom or the number of independent comparisons) and $t = 1{\cdot}13$, p (the probability of falling outside $\pm t$) is between 0·3 and 0·2 and consequently the difference observed between the means of the two weeks is not significant, since statisticians do not recognize as significant any difference for which P is greater than 0·05. Differences of this kind are considered to be due to random sampling.

Example 2. Comparison of the third with the fourth week of lactation regarding the casein content of milk (see Table III).

Cow no.	3rd week	4th week	d	d^2
1	346	319	+27	729
2	354	341	+13	169
3	394	384	+10	100
4	386	370	+16	256
5	391	357	+34	1156
6	400	394	+ 6	36
Totals	2271	2165	+106	2446
Means	378·5	360·8	+17·6	407·66

Variance of the differences	97·90
Variance of the mean of the differences	19·58
Standard error of the mean of the differences ...	4·40
t	4·00
Number of degrees of freedom	5·00
P ... ... smaller than	0·02

and consequently the difference observed between the means of the two weeks is significant.

This arithmetical procedure was carried out for each of the Tables II, III and IV, with the following results:

(*A*) *Total protein nitrogen.*

(1) A regular and highly significant decrease in the percentage of total protein nitrogen was observed from the beginning until the fourth week of lactation.

(2) From the fourth week towards the end of lactation the mean differences of the two consecutive weeks were sometimes positive, and sometimes negative. Tested by "Student's" method, these were sometimes significant and sometimes not significant; no conclusion could therefore be legitimately drawn.

If there was a regular trend after the fourth week, but one which was too small to be readily detected with the number of degrees of freedom present and with the comparison of consecutive week values, it might, nevertheless, be possible to detect it by comparing the figures for every second week or for longer intervals, since the effect of the trend would necessarily be cumulative. The adoption of this process led to the conclusion that there is a small and regular increase in the percentage of total protein nitrogen from the fourth week until the end of the lactation, this increase being more pronounced towards the end of lactation.

Owing to the fact that "tests of significance, in so far as they are accurately carried out, are bound to agree"(8), whatever process of statistical analysis is employed, we attacked the problem from another angle. The mean and the standard error of the mean of each column were computed, and $\pm$ double the standard error was taken as a critical boundary, the corresponding values being plotted on graph paper. By this means, a "band" (i.e. the estimated 95% probability range of the means of columns) was obtained for the whole lactation time. The trend of this "band" at different regions was in perfect agreement with the above conclusion. Table I of the Appendix contains the number of entries, mean, standard error of the mean, and the values $\overline{m}-2\sigma_{\overline{m}}$ and $\overline{m}+2\sigma_{\overline{m}}$ for each column, and the "band" is shown in Fig. 1.

(3) In comparing the lactation curves for the percentage of total protein nitrogen and for the yield of milk we observed that the variations of these two curves were generally in opposite directions (Fig. 2). This suggested that a study of the correlation between the yield and the percentage of total protein nitrogen and also of the variations of the *amount* of total protein nitrogen produced during the lactation time would be worth while.

The correlation coefficient between the yield and the percentage of total protein nitrogen determined for 368 samples of milk was:

$$r = -0{\cdot}454 \pm 0{\cdot}028, \qquad \text{.....(1)}$$

which is clearly significant.

Fig. 3 shows the corresponding correlation table and regression lines. In this table the mean, the standard deviation, and the coefficient of variation are given for each variate. The probable errors given in this table and all others are computed from Karl Pearson's tables(9).

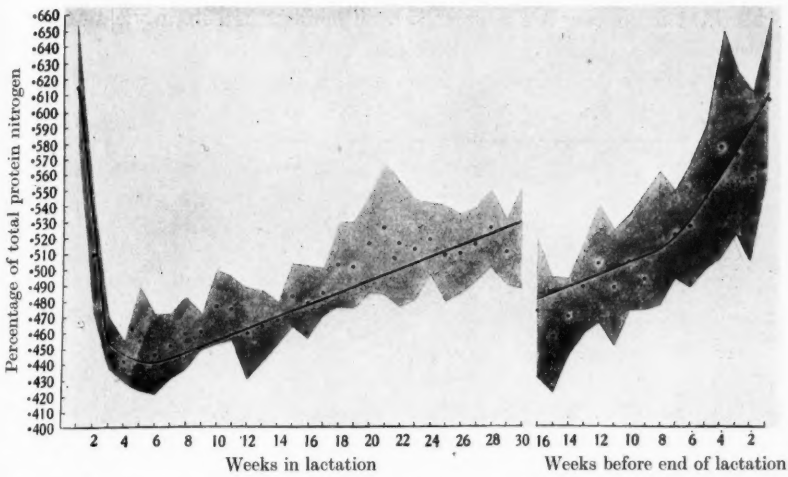

Fig. 1. Showing the lactation curve for the percentage of total protein nitrogen. Each dot represents the mean value of the figures available for the corresponding week of lactation. The "band" shows the limits of $\bar{m} \pm 2\sigma_{\bar{m}}$, i.e. the estimated 95% probability range of the mean values. The length of lactation being different for different cows, the lactation curve is studied in two sections corresponding to the first 30 and the last 16 weeks of lactation.

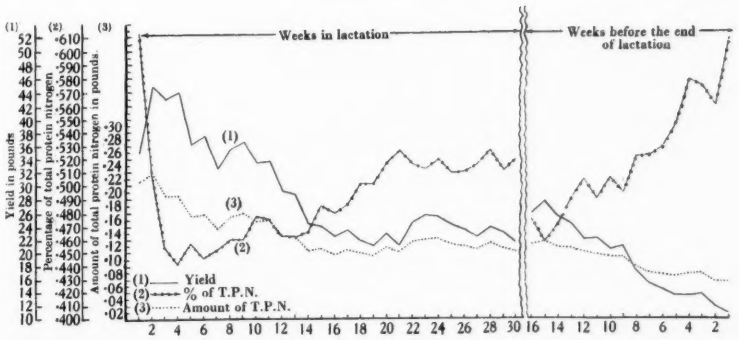
Weeks in lactation
Weeks before the end of lactation
Yield in pounds
Percentage of total protein nitrogen
Amount of total protein nitrogen in pounds
(1) Yield
(2) % of T.P.N.
(3) Amount of T.P.N.

Fig. 2. Showing lactation curves for the yield of milk (1), percentage (2) and the amount (3) of total protein nitrogen in 368 samples of milk taken from twenty-seven individual cows at different stages of lactation. Ordinates of graphs (1) and (2) represent the mean values of the figures for all samples taken in each week of lactation. Ordinates of graph (3) are obtained by multiplying the corresponding ordinates of graphs (1) and (2).

It will be seen from this table:

(1) That the distribution is not normal for the two variates.

(2) That if the cow is a poor milker, an increase of 1 lb. in yield of milk corresponds to a relatively high decrease in the percentage of total protein nitrogen, whereas this decrease is much smaller if the cow is a good milker.

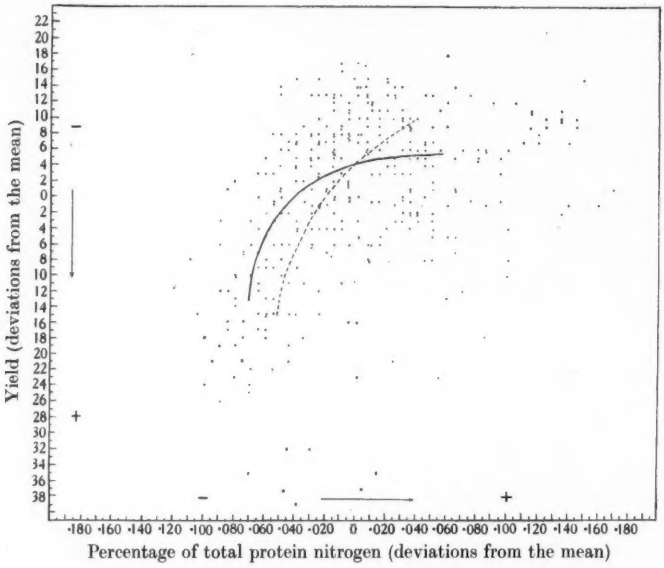

Fig. 3. Showing the correlation between the yield of milk and the percentage of total protein nitrogen in 368 samples of milk taken from twenty-seven individual cows at different stages of lactation. The arrows indicate the direction in which each variate increases. The curve — represents the regression line of the percentage of total protein nitrogen on the yield of milk. The curve ---- represents the regression line of the yield of milk on the percentage of total protein nitrogen. The following statistics indicate the details concerning each variate:

	Yield of milk (lb.)	% of total protein nitrogen
Mean	25·35 ±0·39	0·5095 ±0·0019
Standard deviation	10·98 ±0·27	0·0555 ±0·0014
Coefficient of variation	43·30 ±1·26	10·8000 ±0·3000
Correlation coefficient	$r = -0·454 \pm 0·028$	

The amount of total protein nitrogen produced in each case was the product of milk yield and the corresponding percentage of total protein nitrogen. The curve No. 3 (Fig. 2) which represents the variations of these products shows a constant parallelism with the lactation curve for the yield of milk. This means that with a greater production of milk there is a greater production of proteins; and this in spite of the decrease observed when the proteins are expressed in terms of percentage. The results of the study of the correlation between the yield and the amount of total protein nitrogen produced is in almost perfect

agreement with the above conclusion. The correlation coefficient for the 368 samples of milk examined is:

$$r = 0{\cdot}951 \pm 0{\cdot}003, \qquad \ldots\ldots(2)$$

which is very high.

The two regression coefficients are:

*Regression of milk yield on the amount of total protein nitrogen:

$$0{\cdot}951 \times \frac{10{\cdot}98}{50{\cdot}3} = 0{\cdot}207. \qquad \ldots\ldots(3)$$

*Regression of the amount of total protein nitrogen on yield:

$$0{\cdot}951 \times \frac{50{\cdot}3}{10{\cdot}98} = 4{\cdot}35. \qquad \ldots\ldots(4)$$

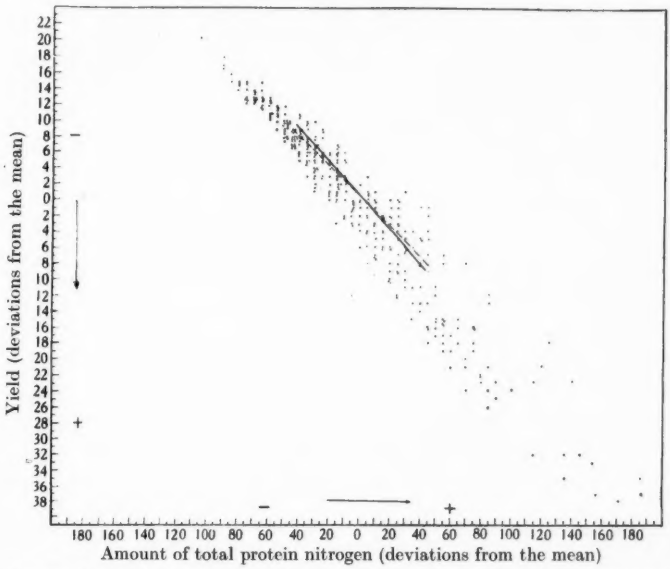

Fig. 4. Showing the correlation between the yield of milk and the yield of total protein nitrogen in 368 samples of milk taken from twenty-seven individual cows at different stages of lactation. The arrows indicate the direction in which each variate increases. The curve — represents the regression line of the yield of milk on the yield of total protein nitrogen. The curve ---- represents the regression line of the yield of total protein nitrogen on the yield of milk. These two curves are not linear; but in the section indicated on the graph they are very near to straight line and may be assumed to be so for practical purposes.

The following statistics indicate the details concerning each variate.

	Yield of milk (lb.)	Yield of total protein nitrogen (1/1000ths of lb.)
Mean	$25{\cdot}35 \pm 0{\cdot}39$	$126{\cdot}3 \pm 1{\cdot}80$
Standard deviation	$10{\cdot}98 \pm 0{\cdot}27$	$50{\cdot}3 \pm 1{\cdot}20$
Coefficient of variation	$43{\cdot}30 \pm 1{\cdot}26$	$39{\cdot}8 \pm 1{\cdot}14$
Correlation coefficient	$r = 0{\cdot}951 \pm 0{\cdot}003$	

* These two regression lines are assumed to be linear. This assumption is not very far from the truth for practical purposes, for all the dots are placed within a narrow band.

The equation (3) means that for an increase of 453·59 g. (1 lb.) in milk there is an increase of about 0·2 mg. in the amount of total protein nitrogen.

Further, equation (4) shows that if the amount of total protein nitrogen rises by 1 mg., there will be an increase of about 4·35 lb. in the yield of milk.

The fact that an increase in yield of milk means an increase in the amount of total protein, and also that an increase in the latter corresponds to an increase in the yield of milk, is of physiological and economic importance. It shows that the synthesis of proteins in milk is not an independent process, but is closely related to the synthesis of the milk itself. In other words it would be impossible to get cows to produce milk with a very low or a very high protein content by manipulation of the environment.

Morris & Wright have studied the biological value of various protein foods for milk production and have come to the conclusion that those containing a relatively high content of lysine and tryptophane are more adequate for milk production than others, since the milk itself contains a large amount of these amino acids and the animal organism cannot produce them (10).

From the conclusion reached above it appears that if the amount of protein in the ration is diminished to such extent as to exhaust the body protein reserve, the result would be not a milk poorer in protein, but a smaller quantity of milk (equation (2)), richer in protein (equation (1)). On the other hand, an increase in the amount of protein in the food would increase the yield if the production of milk were below its maximum level, otherwise it would not make any difference either in the yield or in the percentage of protein in milk.

Perkins (11), Morris (12), and Turner (13) have studied in carefully designed experiments the influence of the amount of protein in food on the yield and on the chemical composition of milk, and their results are in perfect agreement with the above conclusions. Of special interest in this respect is the work of Gowen *et al.* (14). They studied the variations which occurred in the yield and in the composition of the milk of cows receiving no food but having access to as much water as they wished. They observed a progressive and marked decrease in the yield and in the percentage of lactose, and an increase in fat, ash, and protein.

During the present investigation, the writer has also observed an increase in the percentage of total protein nitrogen in the milk of cows whose yield fell during sexual excitement. It is noteworthy that from the analysis of the results of this investigation, which was not concerned with rationing, it has been possible to arrive at the same conclusions as those reached by other investigators who have carried out experiments on the feeding of cattle.

(*B*) *Casein nitrogen.*

The casein nitrogen forms about 77 % (15) of the total protein nitrogen in milk, and the above discussion on total protein nitrogen was found to be

applicable to the casein nitrogen. The same methods of statistical analysis and tests of significance were used and the following results were obtained:

(1) Lactation curve for the percentage of casein nitrogen (Fig. 5) with the

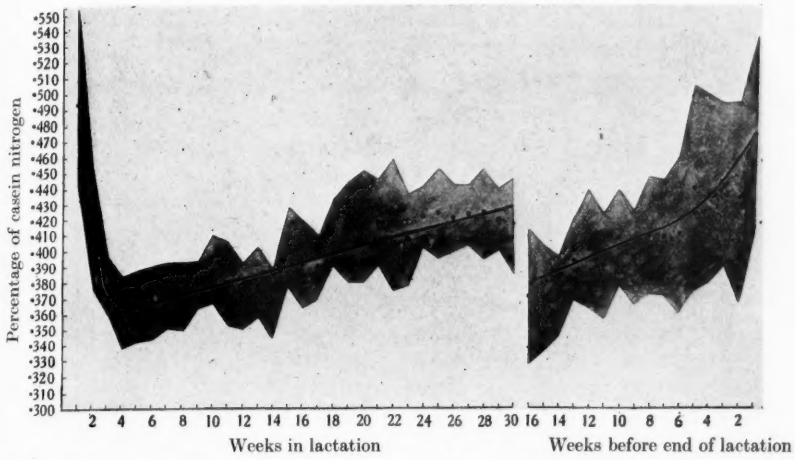

Fig. 5. Showing the lactation curve for the percentage of casein nitrogen. Each dot represents the mean value of all figures available for the corresponding week of lactation. The "band" shows the limits of $\bar{m} \pm 2\sigma_{\bar{m}}$, i.e. the estimated 95% probability range of the mean values.

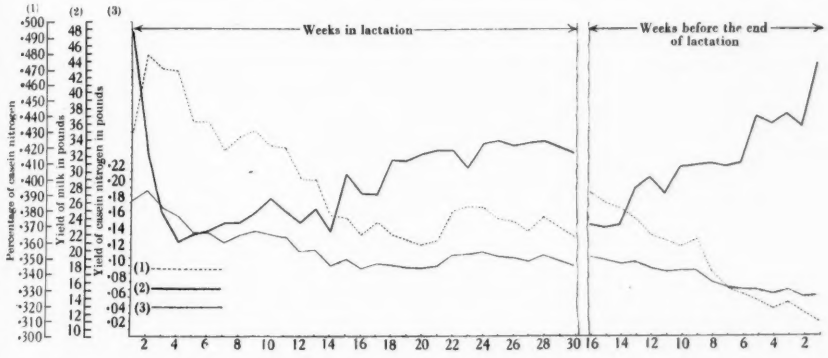

Fig. 6. Showing lactation curves for the yield of milk (1), percentage (2) and the amount (3) of casein nitrogen (363 samples). Ordinates of graphs (1) and (2) represent the mean values of all the figures corresponding to samples taken in each week of lactation. Ordinates of graph (3) are obtained by multiplying the corresponding ordinates of graphs (1) and (2).

corresponding table (Appendix, Table II) containing details of 95% probability range of the estimation of means of columns.

(2) Lactation curve for the amount of casein nitrogen (Fig. 6).

(3) Correlation coefficient between yield and the percentage of casein nitrogen was: $r = -0{\cdot}318 \pm 0{\cdot}031$.

Tocher (16) working with a different material (474 samples from one milking of 474 cows scattered over the whole area of Scotland, and over a whole year), and with a different method of chemical analysis, has found a higher value, i.e. −0·4219.

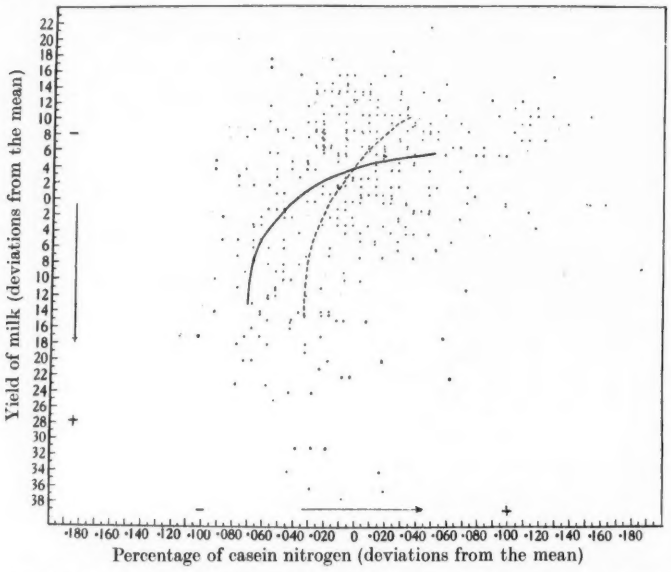

Fig. 7. Showing the correlation between yield of milk and percentage of casein nitrogen in 363 samples of milk. The arrows indicate the direction in which each variate increases. The curve — represents the regression line of the percentage of casein nitrogen on the yield of milk. The curve ---- represents the regression line of the yield of milk on the percentage of total protein nitrogen. The following statistics indicate the details concerning each variate:

	Yield of milk (lb.)	% of casein nitrogen
Mean	25·15 ± 0·39	0·4119 ± 0·0018
Standard deviation	11·03 ± 0·28	0·0511 ± 0·0013
Coefficient of variation	43·80 ± 1·30	12·3000 ± 0·3000
Correlation coefficient	$r = -0·318 \pm 0·031$	

(4) Correlation coefficient between the yield of milk and the yield of casein nitrogen was

$$r = 0·927 \pm 0·0049,$$

and the regression equations

$$\frac{R \text{ yield of milk}}{\text{yield of casein nitrogen}} = 0·927 \times \frac{11·03}{41·9} = 0·244.*$$

$$\frac{R \text{ yield of casein nitrogen}}{\text{yield of milk}} = 0·927 \times \frac{41·9}{11·03} = 3·521.*$$

Figs. 7 and 8 show the corresponding correlation tables with the details concerning each variate.

* See footnote, p. 133.

(*C*) *Albumin and globulin nitrogen.*

(1) The lactation curve (Fig. 9) for the percentage of albumin + globulin nitrogen is of the same general trend as for the casein nitrogen, although the trend for the former is much smaller than for the latter.

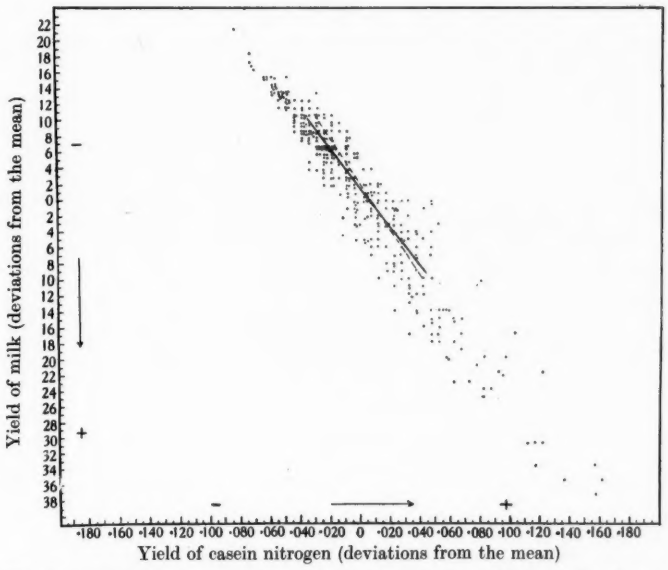

Fig. 8. Showing correlation between the yield of milk and the yield of casein nitrogen in 363 samples of milk taken from twenty-seven individual cows. The arrows indicate the direction in which each variate increases. The curve — represents the regression line of the yield of casein nitrogen on the yield of milk. The curve ---- represents the regression line of the yield of milk on the yield of casein nitrogen. The two curves are not linear but are very near to straight line. The following statistics indicate details concerning each variate:

	Yield of milk (lb.)	Yield of casein nitrogen (1/1000ths of lb.)
Mean	25·15 ± 0·39	101·9 ± 1·50
Standard deviation	11·03 ± 0·28	41·9 ± 1·04
Coefficient of variation	43·80 ± 1·30	41·1 ± 1·20
Correlation coefficient	$r = 0{\cdot}927 \pm 0{\cdot}0049$	

(2) The lactation curve for the amount of albumin and globulin nitrogen is given in Fig. 10.

(3) The correlation table (Fig. 11) between the yield of milk and the percentage of albumin + globulin nitrogen shows that the distribution is very different from that of the casein nitrogen. The coefficient of variability is also much larger than for the casein nitrogen. The correlation coefficient is

$$r = -0{\cdot}274 \pm 0{\cdot}033.$$

Tocher, with the same material as mentioned above, found $r = -0{\cdot}2948$.

(4) The correlation table (Fig. 12) for the yield of milk and the yield of albumin + globulin nitrogen shows a much more scattered distribution than in the case of casein nitrogen. The correlation coefficient is

$$r = 0{\cdot}797 \pm 0{\cdot}013.$$

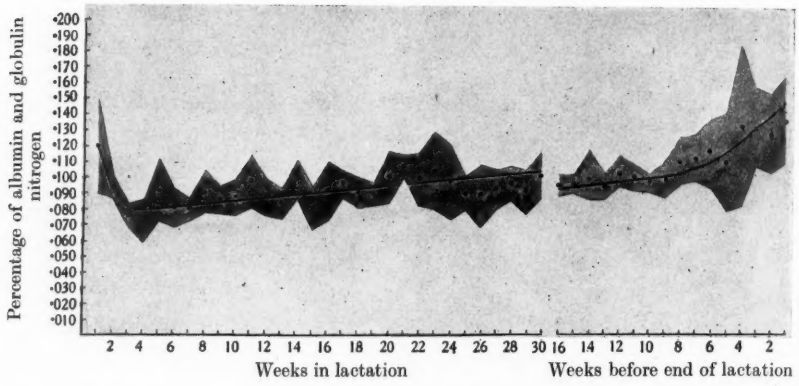

Fig. 9. Showing lactation curve for the percentage of albumin and globulin nitrogen. Each dot represents the mean value of all the figures available for the corresponding week of lactation. The "band" shows the limits of $\overline{m} \pm 2\sigma_{\overline{m}}$, i.e. the estimated 95 % probability range of the mean values.

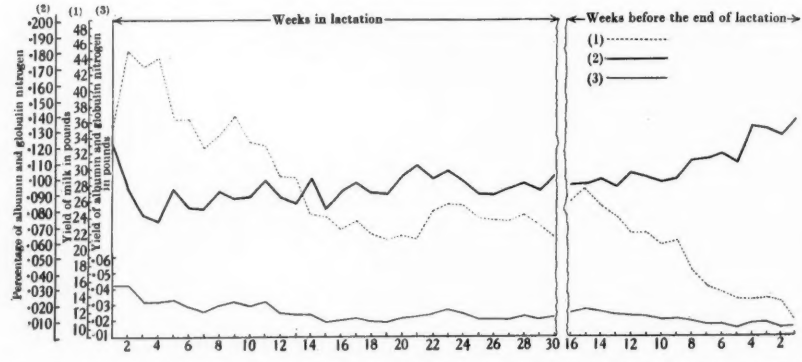

Fig. 10. Showing lactation curves for the yield of milk (1), the percentage (2) and the amount (3) of albumin and globulin nitrogen (356 samples). Ordinates of graphs (1) and (2) represent the mean values of the figures for all samples taken in each week of lactation. Ordinates of graph (3) are obtained by multiplying the corresponding ordinates of graphs (1) and (2).

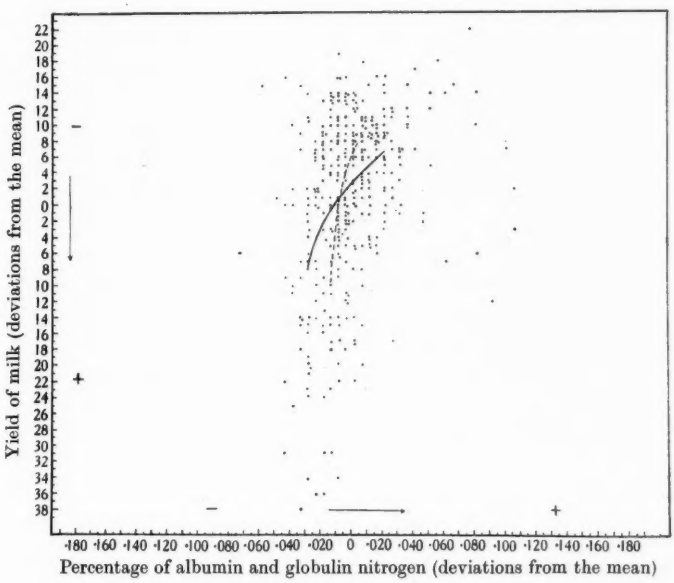

Fig. 11. Showing correlation between the yield of milk and the percentage of albumin and globulin nitrogen (356 samples). The arrows indicate the direction in which each variate increases. The curve — represents the regression line of the percentage of albumin and globulin nitrogen on the yield of milk. The curve ---- represents the regression line of the yield of milk on the percentage of albumin and globulin nitrogen. The following statistics indicate the details concerning each variate:

	Yield of milk (lb.)	Percentage of albumin + globulin nitrogen
Mean	25·26 ± 0·38	0·0973 ± 0·0011
Standard deviation	10·86 ± 0·27	0·0302 ± 0·0007
Coefficient of variation	43·00 ± 1·27	31·0000 ± 0·8500
Correlation coefficient	$r = -0·274 \pm 0·033$	

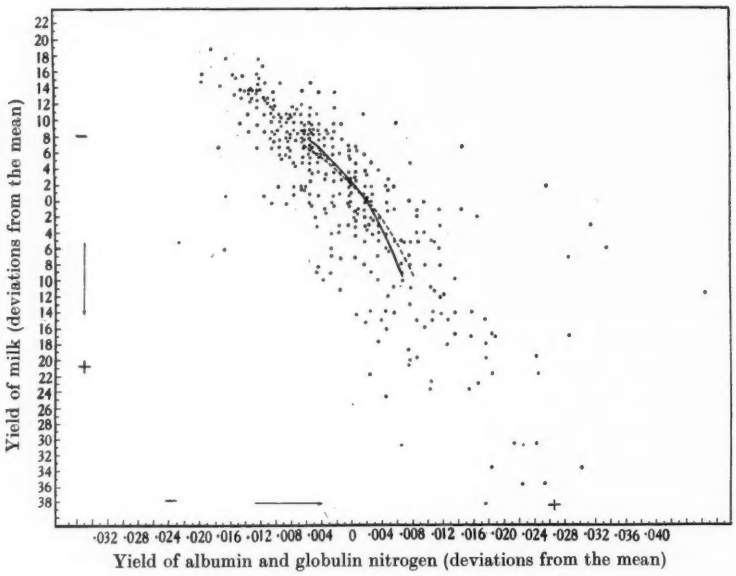

Fig. 12. Showing correlation between the yield of milk and the yield of albumin and globulin nitrogen (356 samples). The arrows indicate the direction in which each variate increases. The curve — represents the regression line of the yield of milk on the yield of albumin and globulin nitrogen. The curve ---- represents the regression line of the yield of albumin and globulin nitrogen on the yield of milk. The following statistics indicate details concerning each variate:

	Yield of milk (lb.)	Yield of albumin + globulin nitrogen (1/1000ths lb.)
Mean	25·26 ± 0·38	23·60 ± 0·36
Standard deviation	10·86 ± 0·27	10·14 ± 0·27
Coefficient of variation	43·00 ± 1·27	42·96 ± 1·27
Correlation coefficient	$r = 0{\cdot}797 \pm 0{\cdot}013$	

DISCUSSION

(1) Bartlett (17), in a study of the "Variations in the solids-not-fat content of milk", states, in referring to "about 40 days after calving", that "for several months after this time the composition of the milk tends to be remarkably constant". The conclusions drawn from the present work do not, however, support this statement. Tocher (16) has shown that there exists a positive and significant correlation between the yield of milk and the percentage of lactose. Since the yield of milk decreases regularly "from about 40 days after calving" towards the end of lactation, one must conclude that the percentage of lactose decreases also in the same direction. The fact observed by Bartlett that the solids-not-fat content of milk remains constant during several months does not necessarily mean that the composition of the milk is also "remarkably constant" during that time. All we can conclude is that the decrease in the percentage of lactose is counterbalanced by the increase in the percentage of proteins and ash (Tocher) in such a way that the percentage of solids-not-fat remains constant.

(2) It is necessary to bear in mind that the facts observed and the conclusions drawn in the present investigation are of a relative value and are by no means strictly applicable to individual cows. In fact, this work, like most of the investigations in biological sciences, is based upon principles which themselves are of a relative value. Consequently the results obtained do not necessarily represent the absolute truth.

The present state of knowledge about the chemistry of casein is very limited. In precipitating casein by Moir's method it is supposed that all the samples of milk have the same initial *p*H. This is not true, since variability is one of the most important characteristics of living organisms, and as Duclaux pointed out: "Il n'y a pas 'du lait' mais bien 'des laits'" (18).

Further, it must be remembered that the principles of statistics are based upon the "law of large populations which 'suivant l'expression d'un grand mathématicien est toujours vraie en général et fausse en particulier'" (19). For instance, just as the length of lactation is different with various cows, the time in which the percentage of various proteins in milk decreases may be different from cow to cow and last from 3 to 6 weeks according to the breed of the cow and other factors.

(3) Actually, from a purely scientific standpoint, this work is no more than a record of a number of measurements from which certain conclusions are drawn. It does not tell us anything about the real causes of the variations observed.

Owing to the exceptional conditions under which these experimental herds are kept, it is probable that the average herd would show somewhat greater variation, and studies of the comparisons of yields of animals from several herds would show still greater variation owing to differences in nutrition and other environmental factors.

Summary

1. It was planned to study the variations of the protein content of milk during the lactation period.

2. The total protein nitrogen and the casein nitrogen were determined by approved methods in about 380 weekly samples of milk taken from twenty-seven individual cows of different breeds and at different stages of lactation, from February to July 1937. The albumin+globulin nitrogen was calculated in each case by difference.

3. The statistical analysis of the figures was made by the methods of "factorial arrangement" and by "pairing", and also by the estimation of the 95% probability range of the mean of the figures available for each week of lactation.

4. It was found that the percentage of total protein nitrogen decreases very significantly from the beginning until the 4th week of lactation, and then rises slowly until the end of lactation, the rise being more pronounced towards the end. The same was true for casein nitrogen and for albumin+globulin nitrogen, but with the latter only the decrease at the beginning and the rise at the end were sharp. The rise for the rest of the lactation curve, although it did exist, was very slow.

5. The lactation curves for the yield of total protein nitrogen, casein nitrogen and albumin+globulin nitrogen were practically parallel with the lactation curve for the yield of milk.

6. The correlations between the yield of milk and the percentages of total protein nitrogen, casein nitrogen and albumin+globulin nitrogen are studied and the correlation tables with all details are given for each. It is noteworthy that the regression lines are not linear and that with a poor milker an increase of 1 lb. in yield of milk corresponds to a relatively high decrease in the percentage of protein nitrogen, whereas with a high milker this decrease is relatively much smaller.

7. The correlations between the yield of milk and the yield of total protein nitrogen, casein nitrogen and albumin+globulin nitrogen are examined and the correlation tables are given. It is shown that there is a close correlation between the yield of milk and its protein content, from which the conclusion is drawn that a very low protein food does not decrease the percentage of protein in milk as one might expect, but decreases the yield of milk and increases the percentage of protein in milk. On the other hand, an increase of adequate protein in the ration may raise the yield of milk if it is not at its maximum level.

8. It is emphasized that the above conclusions are drawn from a study carried out on a group of cows, and may not be strictly applicable to individual cows.

ACKNOWLEDGEMENTS

The writer wishes to acknowledge his gratitude to Prof. F. A. E. Crew for the hospitality and facilities received at the Institute of Animal Genetics during this investigation. He also wishes to express his indebtedness to Mr A. D. Buchanan Smith and Dr A. M. Smith for their valuable help, encouragement and criticism throughout the work. His sincere thanks are also due to Prof. R. A. Fisher and Dr A. C. Aitken for their helpful assistance and advice during the statistical analysis of the work.

APPENDIX

Table I. *Total protein nitrogen*

Estimation of 95% probability range of the mean of the figures available for each week of lactation.

n = number of cows. m = mean. σ_m = standard error of the mean.

Week	n	$\sigma_{\bar{m}}$	$\bar{m} - 2\sigma_{\bar{m}}$	$\bar{m}$	$\bar{m} + 2\sigma_{\bar{m}}$
1	4	20·42	573·2	614·0	654·8
2	4	18·36	472·8	509·5	546·2
3	6	8·43	437·6	454·5	471·4
4	5	6·74	428·1	441·6	455·1
5	8	16·80	423·4	457·0	490·6
6	9	12·88	420·7	446·5	472·3
7	8	10·48	430·7	451·7	472·7
8	10	11·62	437·1	460·4	483·6
9	9	5·19	449·8	468·2	470·6
10	10	11·73	453·3	476·8	500·3
11	11	9·98	455·5	475·5	495·5
12	9	14·56	430·5	459·6	488·7
13	8	11·52	441·1	464·1	487·1
14	6	6·82	452·5	466·1	479·7
15	8	9·91	463·9	483·7	503·6
16	7	11·53	456·2	479·3	502·3
17	8	7·78	469·8	485·4	501·0
18	8	13·30	476·4	503·0	529·6
19	6	14·18	473·6	502·0	530·4
20	7	15·40	485·5	516·3	547·1
21	10	21·18	483·3	525·7	568·1
22	10	19·92	476·2	516·1	555·9
23	10	15·97	480·3	512·3	544·2
24	11	11·62	496·0	519·3	542·5
25	11	15·19	478·8	509·2	539·6
26	12	12·68	484·6	510·0	535·4
27	12	11·59	492·5	515·7	538·9
28	11	12·54	501·3	526·4	551·4
29	11	10·44	490·0	510·9	531·8
30	11	15·78	488·0	519·6	551·2
Weeks before end of lactation					
16	6	21·95	430·7	474·6	518·5
15	6	18·4	421·5	458·3	495·1
14	8	12·3	445·6	470·2	494·8
13	8	14·71	459·4	488·8	518·2
12	9	18·83	466·1	503·8	541·5
11	9	18·6	451·4	488·6	525·8
10	10	15·19	474·3	504·7	535·1
9	9	9·87	474·5	494·3	514·0
8	7	21·6	477·5	520·7	563·9
7	7	14·43	493·6	522·5	551·4
6	7	19·87	488·5	528·2	567·9
5	5	23·1	499·8	546·0	592·2
4	3	35·91	506·2	578·0	649·8
3	6	25·62	521·8	573·0	624·2
2	6	26·68	504·7	558·1	611·5
1	5	25·76	557·9	609·0	660·5

Table II. *Casein nitrogen*

Estimation of 95% probability range of the mean of the figures available for each week of lactation.

n = number of cows. m = mean. σ_m = standard error of the mean.

Weeks	n	$\sigma_{\bar{m}}$	$\bar{m} - 2\sigma_{\bar{m}}$	$\bar{m}$	$\bar{m} + 2\sigma_{\bar{m}}$
1	4	25·9	442·2	494·0	545·8
2	4	19·9	375·95	415·75	455·55
3	6	9·2	360·1	378·5	396·9
4	6	11·4	338·03	360·83	383·63
5	8	11·7	341·6	365·0	388·4
6	8	11·7	344·22	367·62	391·02
7	9	10·5	350·62	371·62	392·62
8	9	10·9	349·6	371·4	393·2
9	10	7·3	364·4	379·0	393·6
10	10	10·7	367·0	388·4	409·8
11	10	13·5	353·3	380·3	407·3
12	9	10·5	351·2	372·2	393·2
13	8	11·2	358·22	380·62	403·02
14	6	10·8	345·4	367·0	388·6
15	8	13·2	376·6	403·0	429·4
16	8	13·7	363·97	391·37	418·77
17	8	10·3	369·27	389·87	410·47
18	8	10·9	390·7	412·5	434·3
19	5	16·3	379·9	412·5	445·1
20	7	18·1	379·8	416·0	452·2
21	10	13·6	391·2	418·4	445·6
22	10	20·7	376·2	417·6	459·0
23	10	14·9	377·9	407·7	437·5
24	11	10·0	401·81	421·81	441·81
25	10	14·3	395·4	424·0	452·6
26	12	11·1	399·1	421·3	443·5
27	12	10·2	402·68	423·08	443·48
28	10	14·5	395·4	424·4	453·4
29	11	10·0	399·9	419·9	439·9
30	10	15·4	385·2	416·0	445·8
Weeks before end of lactation					
16	7	22·2	326·6	371·0	415·4
15	7	16·7	336·0	369·4	402·8
14	8	12·1	357·3	371·5	395·7
13	8	13·4	367·8	394·6	421·4
12	9	19·0	363·0	401·0	439·0
11	10	16·7	357·4	390·8	424·2
10	10	15·6	376·7	407·9	439·1
9	9	15·4	364·7	395·5	426·3
8	7	19·0	372·0	410·0	448·0
7	6	18·7	371·2	408·6	446·0
6	6	25·1	360·4	410·6	460·8
5	4	33·2	373·6	440·0	506·4
4	4	27·9	379·9	435·7	491·5
3	6	26·2	389·6	442·0	494·4
2	6	32·3	366·2	430·8	495·4
1	5	30·5	412·6	473·6	534·6

Table III. *Albumin nitrogen + globulin nitrogen*

Estimation of 95% probability range of the mean of the figures available for each week of lactation.

n = number of cows. m = mean. σ_m = standard error of the mean.

Week	n	$\sigma_{\overline{m}}$	$\overline{m} - 2\sigma_{\overline{m}}$	$\overline{m}$	$\overline{m} + 2\sigma_{\overline{m}}$
1	4	14·7	90·3	119·7	149·1
2	4	3·2	86·85	93·25	99·65
3	6	3·7	68·6	76·0	83·4
4	5	6·9	58·6	72·4	86·2
5	8	9·4	73·45	92·25	111·05
6	8	6·0	69·0	81·0	93·0
7	8	3·4	73·2	80·0	86·8
8	9	6·3	78·4	91·0	103·6
9	9	4·0	78·2	86·2	94·2
10	10	5·6	76·8	88·0	99·2
11	10	8·2	81·2	97·6	114·0
12	9	5·3	76·4	87·0	97·6
13	8	4·9	73·82	83·62	93·42
14	6	5·8	87·2	99·0	110·8
15	8	6·7	67·1	80·5	93·9
16	7	9·0	73·42	91·42	109·42
17	8	4·3	87·02	95·62	104·02
18	8	4·9	80·7	90·5	100·3
19	5	3·7	81·8	89·2	96·6
20	6	8·2	83·76	100·16	116·56
21	10	3·7	100·0	107·4	114·8
22	11	8·4	82·01	98·81	115·61
23	10	12·4	79·9	104·7	129·5
24	11	11·8	73·76	97·36	120·96
25	10	5·1	79·2	89·4	99·6
26	12	9·6	69·3	88·5	107·7
27	12	6·2	80·1	92·5	104·9
28	10	5·2	85·9	96·3	106·7
29	11	7·1	76·89	91·09	105·29
30	10	7·3	86·7	101·3	115·9
Weeks before end of lactation					
16	6	3·6	87·9	95·1	102·3
15	6	2·9	90·3	96·1	101·9
14	8	6·4	86·0	98·8	111·6
13	8	3·9	86·4	94·2	102·0
12	9	4·4	93·8	102·6	111·4
11	9	4·4	91·3	100·1	108·9
10	10	3·1	90·4	96·6	102·8
9	9	6·1	86·8	99·0	111·2
8	7	7·5	95·5	110·5	125·5
7	6	7·5	96·8	111·8	126·8
6	6	11·2	92·7	115·1	137·5
5	4	15·5	78·5	109·5	140·5
4	3	25·4	81·2	132·0	182·8
3	6	12·3	106·4	131·0	155·6
2	6	12·8	101·7	127·3	152·9
1	5	13·4	109·0	135·8	162·6

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636.2:612.664.3:519.24

183. ERRORS INVOLVED IN THE ESTIMATION OF THE LACTATION YIELD OF PROTEIN ACCORDING TO THE INTERVALS BETWEEN SAMPLING

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Variations in protein yield of a cow are due to changes both in her yield of milk and in the percentage of protein in her milk. If it were possible to have, for each day of lactation, figures for the yield of milk and its percentage of protein, the estimation of lactation yield of protein would be easy, but in practice this is not possible since the determination of protein in milk is a long and laborious process.

In measuring the yield and the protein content of milk at intervals and taking the figures obtained each time as averages for a whole interval, errors will necessarily be introduced in the estimation of lactation yield of protein. These errors will be due partly to changes in the yield of milk and partly to changes in its protein content. Those due to the former cause may be eliminated if the daily yields of milk are measured and the results used in the calculation of protein yield.

The present investigation (which is to be used as a basis in a series of experiments on cows whose milks are weighed daily) is concerned only with errors due to variations in the protein content. The material used has already been described (1). It is based on the results of analyses of 380 daily samples of milk taken from twenty-seven cows in different stages of lactation at intervals of 7 days. Tocher (2) has shown that in milk the coefficient of variation for casein is much smaller than that for albumin which, in turn, is similar to that for fat. As weekly samples are considered sufficiently reliable for the estimation of total yield of fat, the same was assumed to be true for total protein nitrogen.

The method adopted in this investigation for testing the accuracy of fortnightly, as against weekly, measurement, is based on the degree of error involved in a single test compared with the mean of two consecutive tests, one of which is the figure used for the single test. Likewise, the accuracy of determinations made at intervals of three weeks is obtained by examination of the degree of error involved in a single test compared with the mean of three consecutive tests, one of which is the figure used for the single test. If the mean is taken as equal to 100, then the error of the single figure can be expressed as a percentage of the mean. When a large number of consecutive figures are treated in this manner, a "population" of errors is obtained. The mean of this "population"

is zero, and its standard deviation gives the limits within which 68% of the "population" is found.

For the test of the fortnightly determinations, fifty pairs of two consecutive determinations were taken at random from the data, at least one being included from each of the cows whose milk had been tested (except cow No. 27 whose milk was tested only twice). To test the accuracy of three-weekly determinations, thirty-four groups of three consecutive figures were taken, again at random, including all the cows except Nos. 26 and 27. Tables I and II show the results and the details of calculation of the standard deviation in each set. For fortnightly determinations, the standard deviation is $= \pm 2{\cdot}92$. This means that, by taking weekly samples as the basis of comparison, the use of fortnightly samples will involve errors which will be found within the limits of $\pm 2{\cdot}92\%$ 68 times out of 100, and 95 times out of 100 they will be found within the limits of $\pm 5{\cdot}84$. With three-weekly samples, the standard deviation is slightly greater, i.e. 2·99%, and 95% of errors will fall within the limits of $\pm 5{\cdot}98\%$.

Discussion and conclusion

The present writer(1) has shown that the total protein nitrogen decreases very rapidly during the first 6 weeks of lactation, and thereafter increases slightly until nearly the end of the lactation, when the increase becomes more pronounced. If, in the randomized groups of two or three consecutive weeks, those corresponding to the two periods of rapid change are eliminated, relatively smaller limits of errors are obtained. Thus it is suggested that weekly samples, taken during the first 6 and the last 4 weeks of lactation, with fortnightly samples for the remainder, should give results which would not deviate seriously from those for weekly samples throughout the lactation. For practical purposes, however, a sufficient degree of accuracy would be obtained by fortnightly samples during the periods of rapid change, and samples taken at intervals of three weeks during the remainder of the lactation.

It is emphasized that these results are valid only when the daily yields of milk are known for the whole lactation and used for the calculation of protein yield; if this is not the case, i.e. if the milk is recorded only at intervals, the error will obviously be greater.

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Table I. *Estimation of the errors involved in the determination of lactation yield of protein when two-weekly samples are taken instead of weekly ones, and when the true yields of milk are used in the calculation of protein yield*

Cow no.	Mg. of total protein nitrogen in 100 g. of milk	Means	*d* (absolute values of the differences from the means)	*D* (*d* expressed as % of mean)	D^2
1	421	424·0	3·0	0·71	0·5041
	427				
	471	467·0	4·0	0·09	0·0081
	463				
2	512	474·5	37·5	7·91	62·5681
	437				
	453	461·5	8·5	1·84	3·3856
	470				
3	563	521·5	41·5	7·97	63·5209
	480				
	468	464·0	4·0	0·86	0·7396
	460				
4	464	462·0	2·0	0·43	0·1849
	460				
	462	456·5	5·5	1·20	1·4400
	451				
5	420	435·0	15·0	3·45	11·9025
	450				
	433	468·0	35·0	7·49	56·1001
	503				
6	488	482·0	6·0	1·24	1·5376
	476				
	480	470·5	9·5	2·01	4·0401
	461				
	509	518·5	9·5	1·83	3·3489
	528				
7	457	458·5	1·5	0·33	0·1089
	460				
	484	494·5	10·5	2·12	4·4944
	505				
8	572	551·0	21·0	3·80	14·4400
	530				
	496	507·5	11·5	2·26	5·1076
	519				
9	432	440·0	8·0	1·81	3·2761
	448				
10	436	442·0	6·0	1·36	1·8496
	448				
	492	493·0	1·0	0·20	0·0400
	494				
11	417	433·5	16·5	3·81	14·5161
	450				
12	486	497·0	11·0	2·20	4·8400
	508				
	470	477·0	7·0	1·46	2·1316
	484				
13	513	492·5	20·5	4·16	17·3056
	472				
	457	458·5	1·5	0·33	0·1089
	460				

Table I (*continued*)

Cow no.	Mg. of total protein nitrogen in 100 g. of milk	Means	*d* (absolute values of the differences from the means)	*D* (*d* expressed as % of mean)	D^2
14	679	632·0	47·0	7·43	55·2049
	585				
	545	545·0	0·0	0·00	0·0000
	545				
15	468	481·5	13·5	2·79	7·7841
	495				
	479	506·0	27·0	5·35	28·6225
	533				
	465	478·5	13·5	2·82	7·9524
	492				
16	496	492·5	3·5	0·71	0·5041
	489				
	500	514·5	14·5	2·81	7·8961
	529				
17	524	514·5	9·5	1·84	3·3856
	505				
	500	503·5	3·5	0·70	0·4900
	507				
18	448	454·0	6·0	1·32	1·7424
	460				
	438	436·5	1·5	0·34	0·1156
	435				
19	555	548·5	6·5	1·18	1·3924
	542				
	547	556·5	9·5	1·70	2·8900
	566				
20	527	524·5	2·5	0·47	0·2209
	522				
	538	541·5	3·5	0·65	0·4225
	545				
21	493	504·5	11·5	2·28	5·1984
	516				
	533	537·5	4·5	0·84	0·7056
	542				
22	592	599·0	7·0	1·17	1·3689
	606				
	632	632·5	0·5	0·08	0·0064
	633				
23	652	653·5	1·5	0·23	0·0529
	655				
	601	615·5	14·5	2·35	5·5225
	630				
24	526	538·5	12·5	2·32	5·3824
	551				
25	515	522·5	7·5	1·43	2·0449
	530				
	530	535·5	5·5	1·03	1·0609
	541				
26	644	629·0	15·0	2·38	5·6644
	614				

Total number of degrees of freedom = 99.

$\Sigma D^2 = 846{\cdot}26.$

$$\sigma = \sqrt{\frac{846{\cdot}26}{99}} = \pm 2{\cdot}92.$$

Table II. *Estimation of the errors involved in the determination of lactation yield of protein when three-weekly samples are taken instead of weekly ones and when the true yields of milk are used in the calculation of protein yield*

Cow no.	Mg. of total protein nitrogen in 100 g. of milk	Means	*d* (absolute values of the differences from the means)	*D* (*d* expressed as % of mean)	D^2
1	459	453·00	6·00	1·33	1·7689
	436		17·00	3·76	14·1376
	464		11·00	2·43	5·9049
2	453	460·00	7·00	1·52	2·3104
	470		10·00	2·17	4·7089
	457		3·00	0·65	0·4225
3	465	443·66	21·34	4·80	23·0400
	440		3·66	0·82	0·6724
	426		17·66	3·93	15·4449
	460	497·66	37·66	7·53	56·7000
	523		25·34	5·07	25·7049
	510		12·34	2·47	6·1009
4	440	430·00	10·00	2·32	5·3824
	420		10·00	2·32	5·3824
	430		0·00	0·00	0·0000
	430	426·00	4·00	0·94	0·8836
	408		18·00	4·23	17·8929
	440		14·00	3·29	10·8241
5	503	472·66	30·34	6·40	40·9600
	451		21·66	4·57	20·8849
	464		8·66	1·83	3·3489
6	468	480·33	12·33	2·56	6·5536
	482		1·67	0·35	0·1225
	491		10·67	2·22	4·9284
7	552	552·33	0·33	0·06	0·0036
	550		2·33	0·42	0·1764
	555		2·67	0·48	0·2304
8	546	548·33	2·33	0·42	0·1764
	527		21·33	3·88	15·0544
	572		23·67	4·31	18·5761
	530	507·33	22·67	4·47	19·9809
	481		26·33	5·19	26·9361
	511		3·67	0·72	0·5184
9	448	445·33	2·67	0·60	0·3600
	447		1·67	0·37	0·1369
	441		4·33	0·97	0·9609
	452	460·66	8·66	1·88	3·5344
	467		6·34	1·38	1·9044
	463		2·34	0·51	0·2601
	503	505·33	2·33	0·46	0·2116
	509		3·67	0·73	0·5329
	504		1·33	0·26	0·0676
10	453	474·00	21·00	4·43	19·6249
	509		35·00	7·38	54·4644
	460		14·00	2·95	8·7025
11	446	440·33	5·67	1·29	1·6641
	430		10·33	2·34	5·4756
	445		4·67	1·06	1·1236
	440	435·66	4·34	0·99	0·9801
	418		17·66	4·04	16·3216
	449		13·34	3·05	9·3025
	450	444·33	5·67	1·27	1·6129
	431		13·33	3·00	9·0000
	452		7·67	1·72	2·9584

Table II (*continued*)

Cow no.	Mg. of total protein nitrogen in 100 g. of milk	Means	*d* (absolute values of the differences from the means)	*D* (*d* expressed as % of mean)	D^2
12	526	513·00	13·00	2·53	6·4009
	499		14·00	2·73	7·4529
	514		1·00	0·19	0·0361
13	477	489·66	12·66	2·58	6·6564
	500		10·34	2·11	4·4521
	492		2·34	0·48	0·2304
14	607	601·33	5·67	0·94	0·8836
	589		12·33	2·04	4·1616
	608		6·67	1·11	1·2321
15	492	479·33	12·67	2·65	7·0225
	480		0·67	0·14	0·0196
	466		13·33	2·79	7·7841
16	557	559·33	2·33	0·42	0·1764
	561		1·67	0·30	0·0900
	560		0·67	0·12	0·0144
17	523	533·33	10·33	1·93	3·7249
	540		6·67	1·25	1·5625
	537		3·67	0·69	0·4761
18	506	534·33	28·33	5·30	28·0900
	562		27·67	5·17	26·7289
	535		0·67	0·12	0·0144
19	542	544·33	2·33	0·43	0·1849
	548		3·67	0·68	0·4624
	543		1·33	0·24	0·0576
20	513	528·00	15·00	2·83	8·0089
	517		11·00	2·08	4·3264
	554		26·00	4·91	24·1081
21	511	488·66	22·34	4·58	20·9764
	495		6·34	1·30	1·6900
	460		28·66	5·88	34·5744
22	644	614·66	29·34	4·75	22·5625
	606		8·66	1·40	1·9600
	594		20·66	3·35	11·2225
	606	623·66	17·66	2·83	8·0089
	632		8·34	1·33	1·7689
	633		9·34	1·49	2·2201
23	647	646·00	1·00	0·15	0·0240
	637		9·00	1·39	1·9321
	654		8·00	1·24	1·5376
24	526	542·66	16·66	3·06	9·3636
	562		19·34	3·56	12·6736
	540		2·66	0·49	0·2401
	562	528·33	33·67	6·36	40·4496
	540		11·67	2·20	4·8400
	483		45·33	8·57	73·4449
25	515	528·66	13·66	2·58	6·6564
	530		1·34	0·25	0·0625
	541		12·34	2·33	5·4289

Total number of degrees of freedom = 101.

$\Sigma D^2 = 900{\cdot}9274$.

$$\sigma = \sqrt{\frac{900{\cdot}9274}{101}} = \pm 2{\cdot}99.$$

(*MS. received for publication* 13 *January* 1938)

637.174

184. THE MILK OF THE GOAT UNDER ENGLISH CONDITIONS

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(With 7 Figures)

Introduction

The paucity of reliable information relating to the milk of the goat led to the work described below, which was carried out over the two complete years 1936 and 1937. It appeared desirable to choose a long period in order that the samples of milk examined should be drawn during all stages of lactation. This allowed the effect of advancement of lactation on the composition of the milk to be traced, and also obviated any disturbing effect on the general averages which might be produced by taking an undue proportion of samples at any one stage of the lactation period. Incidentally this was facilitated by the fact that the goat, unlike the cow, has a more or less definite "rutting" and "kidding" season. With some goats, however, the lactation may be prolonged to an unusual extent, for example with one of our animals it has extended over six years and a daily yield of about half a gallon of milk is still maintained.

It was also desirable to reduce as far as possible the effect upon the general averages of such factors as environment and methods of feeding, intervals between milking times and the inclusion of an undue number of samples from one or more breeds. With the co-operation of the British Goat Society a system was evolved whereby samples were taken and submitted for analysis at regular intervals by interested goat-keepers throughout Great Britain. The information supplied with the samples, which included the breed, yield, kidding date, and age of animal, enabled this object to be achieved and allowed of the separate examination of the milk of the various breeds.

The need for reliable information on the yield and composition of the milk of the breeds kept in Great Britain is urgent from the practical, and important from the scientific points of view, for an infusion of new blood has from time to time occurred and at present there is some demand for further influx(1). The economic possibilities of goat's milk also should not lightly be dismissed, for there are large numbers of well authenticated cases of beneficial effects following its use in infant and invalid feeding where cow's milk was not tolerated. The comparative freedom of the goat from tuberculosis and its ability to thrive in some places where cows cannot must always be points in its favour. When it is realized that the world population of goats reaches a very high

figure (2) (probably over 100 millions) and that large increases in yields have followed the development of the goat in this and some other European countries, the potentialities as they affect the world's food supply need no stress.

Methods of analysis employed

Fat. By the Gerber method using butyrometers of N.P.L. tolerance and an electric centrifuge. The results given by the method were compared with results by the Gottlieb method, as the view had been expressed that the Gerber method might give low results with goat's milk owing to the small size of the fat globules. We have found the Gerber method to be quite reliable for goat's milk.

Solids-not-fat. By calculation from specific gravity figures (determined by a reliable hydrometer) and fat percentages, using Richmond's formula. This method is admittedly approximate, but in our experience, as ascertained by gravimetric determination on duplicate samples, the formula is as good for goat's milk as for cow's milk. The results are sometimes higher and sometimes lower than those obtained by the gravimetric method, but on the whole positive and negative errors occur in about equal numbers, so that for the purpose of the general averages given in this paper such errors will not materially influence the findings.

Lactose. By a modification of the iodometric method of Richmond and Ellison using 0·05N iodine solution, the lactose equivalent of which was determined and found to be 0·00841 g. lactose per ml.

Total nitrogen. By Kjeldahl-Gunning method.

Total protein and casein. By Moir's method using the factor 6·38 for conversion of nitrogen into protein.

Albumin and globulin. By difference between total protein and casein percentages.

Non-protein nitrogen. By difference between total nitrogen and total protein nitrogen.

Ash. By ignition of the total solids at low temperature.

Calcium. By oxalate-permanganate titration.

Phosphoric acid. By wet oxidation of the organic matter followed by precipitation with ammonium molybdate and magnesia mixture.

Chloride. By the wet oxidation method of Davies.

The goat populations represented by the samples examined

The numbers of samples of milk analysed, the numbers of animals from each breed and of herds from which these were obtained are shown in Table I.

In the determinations of fat and solids-not-fat, the samples analysed represented the milk of individuals and included evening and morning samples in equal numbers. The more detailed analyses were made on bulked evening and morning samples; the number of animals contributing to such samples

varied but was usually about four. From Table II and the following pages it will be noted that, in the main, the breeds were represented in sufficient numbers and were derived from a sufficient number of herds to allow of sound conclusions being drawn from the data, but difficulty was experienced with the English breed owing to the smaller numbers of this breed and their concentration in so few herds. The data for this breed are, for the sake of completeness, included in the statement of general average composition, but for the above reasons they will not be further discussed and no conclusions will be drawn from them.

Table I. *Numbers of herds, animals and samples*

	Fat and solids-not-fat			Other constituents		
Breed	Samples	Animals	Herds	Samples	Animals	Herds
Anglo-Nubian	440	52	11	10	50	9
Saanen	100	23	10	6	19	5
British Saanen	696	78	23	9	39	5
British	374	55	19	11	46	8
British Alpine	490	78	20	9	42	8
Toggenburg	210	13	4	7	13	4
British Toggenburg	256	40	10	8	21	5
English	96	6	2	3	5	1
All goats	2662	345	99	63	235	45

Table II. *The average composition of goat's milk compared with that of cow's milk*

	Cow's milk	Goat's milk			
	%	%	S.E.	Maximum	Minimum
Fat	(3) 3·67	4·50	0·030	9·70	1·90
Solids-not-fat	(3) 9·02	8·68	0·016	10·90	6·90
Lactose	(3) 4·78	4·08	0·032	4·85	3·61
Total nitrogen × 6·38	(3) 3·42	3·33	0·061	4·46	2·67
Total proteins	(6) 3·23	2·90	0·059	4·15	2·27
Casein	(6) 2·63	2·47	0·050	3·35	1·85
Albumin and globulin	(6) 0·60	0·43	0·027	0·97	0·14
Non-protein nitrogen × 6·38	(6) 0·19	0·44	0·017	0·86	0·20
Ash	(3) 0·73	0·79	0·010	1·00	0·65
Calcium (CaO)	(4) 0·184	0·194	0·003	0·252	0·150
Chloride (Cl)	(5) 0·105	0·154	0·002	0·193	0·107
Phosphoric acid (P_2O_5)	(4) 0·234	0·27	0·010	0·421	0·136
Ratio P_2O_5/CaO	(4) 1·27	1·39			

The average percentage composition of goat's milk as deduced from the available data of earlier workers is fat 4·47, solids-not-fat 9·15, lactose 4·46, ash 0·74. The figures are, however, very old and in many cases relate to the milk of a few animals.

THE AVERAGE COMPOSITION OF GOAT'S MILK COMPARED WITH THAT OF COW'S MILK

Table II is based on data obtained from all samples given in Table I and includes the standard errors of the mean percentages.

In comparison with the milk of the cow, the milk of the goat is on the average richer in fat and poorer in solids-not-fat. The lactose content is lower and, as might be expected, the chloride content is higher. In this connexion it appears worth while to point out that, like the formula for the calculation of

the approximate solids-not-fat content from specific gravity and fat percentage, Davies' formula for calculating lactose content from chloride content $\left(\text{lactose } \% = 7 - \frac{\text{mg. Cl}}{51}\right)$ is but a general statement which may not apply to a specific case, such as the milk from an individual animal. On the general averages given in Table II such a calculated figure agrees very well with the average figure arrived at by determination, viz. 4·02 against 4·08, but taking the sixty-three samples examined as individuals, the calculated figure would, in a large proportion of cases, depart too widely from that determined to allow of much reliance being placed on it. Thus in 60% of the samples examined the error would range from 0·3 to 0·7%. It may be that there is considerable variation in the proportion of the osmotic pressure of goat's milk due respectively to lactose and chloride, and that this accounts for the formula being less dependable.

Table III. *The average distribution of the nitrogen of goat's milk compared with that of cow's milk*

	Cow's milk*	Goat's milk			
	%	%	S.E.	Maximum	Minimum
Total protein	94·5	86·8	0·44	93·2	74·9
Casein	77·0	74·0	0·52	81·8	62·7
Albumin and globulin	17·5	12·8	0·63	29·0	4·1
Non-protein nitrogen	5·5	13·2	0·44	25·1	6·8

* Calculated.

The proportions of calcium and phosphorus tend to be higher, the percentages of casein and albumin and globulin tend to be lower and that of non-protein nitrogen considerably higher, than is found in cow's milk.

The differences in nitrogen distribution are shown in detail in Table III.

The frequencies with which the various fat and solids-not-fat percentages occurred are shown diagrammatically in Figs. 1–4. The results show that 11% of the evening samples differed from the mean fat percentage by not more than 0·2, 38% by not more than 0·6 and 72% by not more than 1·20. For the same variations the figures for morning samples are 15, 42 and 76. As regards the solids-not-fat the composition is much more constant and there is little difference between evening and morning samples. These can therefore be considered together; 25% of the samples differed from the mean solids-not-fat percentage by not more than 0·2, 50% by not more than 0·4 and 83% by not more than 0·8. These deviations from the mean of the fat and solids-not-fat agree fairly well with those calculated from the normal distribution in terms of the standard deviation.

The bearing of these results on any future legislation may perhaps be considered. It will be seen that 3% of the evening and 7·3% of the morning samples failed to reach the legal presumptive standard for cow's milk of 3% fat. As regards solids-not-fat, however, 42% failed to reach the legal presumptive limit of 8·5% solids-not-fat. Such a standard would, therefore,

certainly be too high for goat's milk sold from individual animals. It may be mentioned that a few determinations of freezing point were made during the progress of the work, and that the results agreed with those found for cow's milk, the average figure being −0·55° C.

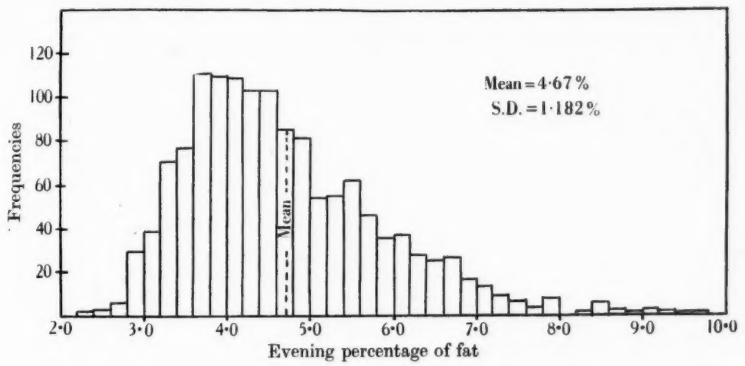

Fig. 1.

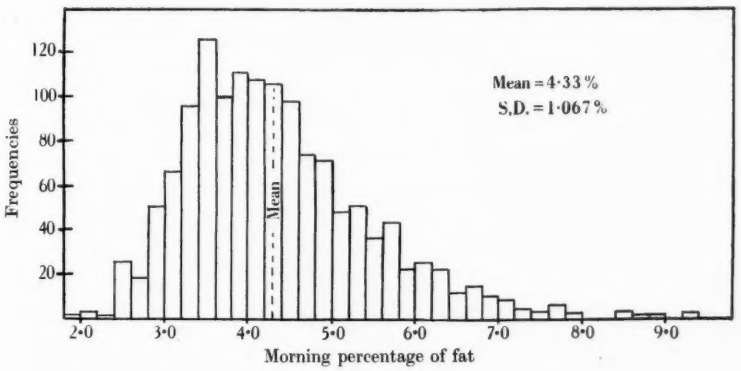

Fig. 2.

COMPARISON OF THE COMPOSITION OF THE MILK OF VARIOUS BREEDS

The average composition of the milk of the various breeds is given in Tables IV and V, where the means and standard errors of the means of each constituent are shown. In addition, the maximum and minimum values and the percentage of samples from each herd falling within specified limits of fat and solids-not-fat are given in Tables VI and VII. The following comments may be made:

(*a*) *Fat and solids-not-fat.* Differences in the fat and solids-not-fat content between the various breeds have been assumed to be significant if the difference between two means exceeded three times the standard error of their difference.

As regards fat content, the milk of the Anglo-Nubian is significantly richer

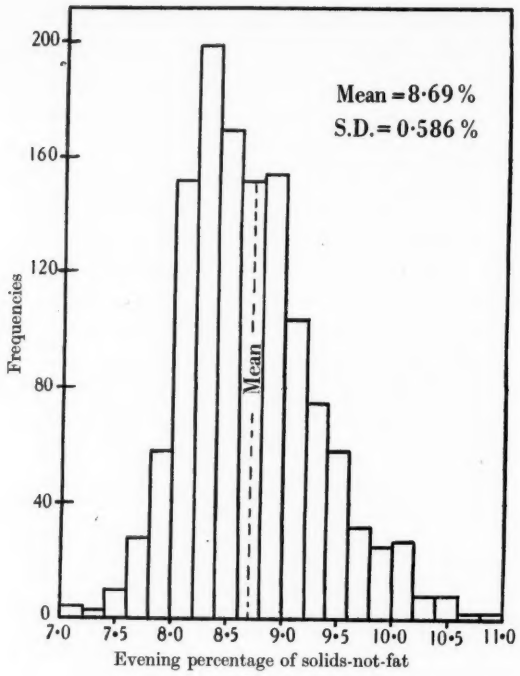
Mean = 8·69 %
S.D. = 0·586 %
Mean
Frequencies
200
160
120
80
40
0
7·0
7·5
8·0
8·5
9·0
9·5
10·0
10·5
11·0
Evening percentage of solids-not-fat

Fig. 3.

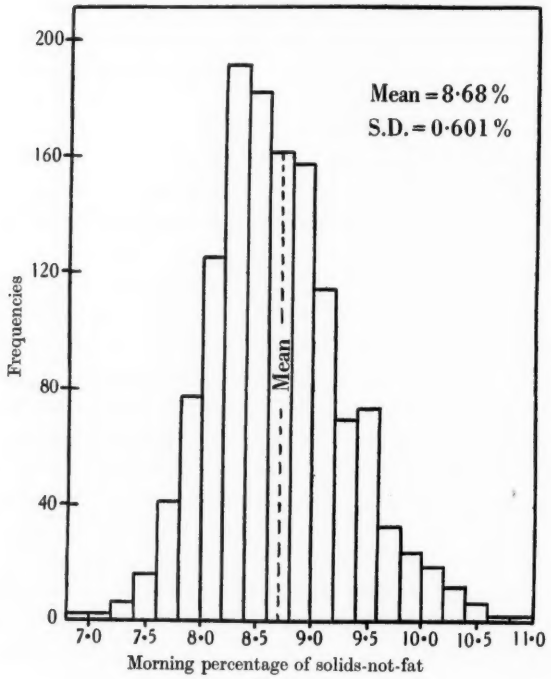
Mean = 8·68 %
S.D. = 0·601 %
Mean
Frequencies
200
160
120
80
40
0
7·0
7·5
8·0
8·5
9·0
9·5
10·0
10·5
11·0
Morning percentage of solids-not-fat

Fig. 4.

than that of all other breeds. The British Toggenburg is significantly richer than the Toggenburg, Saanen and British Saanen. The Toggenburg is significantly poorer than all other breeds on the evening samples, but on morning samples is not significantly poorer than the Saanen, British Saanen and British Alpine. There appears to be no significant difference between the Saanen and British Saanen. With the exception of the Toggenburg the milk of all breeds is significantly higher in fat in the evening than in the morning samples.

Table IV. *Mean percentage of fat and solids-not-fat in the milk of breeds*

	Fat					Solids-not-fat				
	Evening		Morning		Mixed	Evening		Morning		Mixed
	%	S.E.	%	S.E.	%	%	S.E.	%	S.E.	%
Anglo-Nubian	5·94	0·080	5·35	0·076	5·60	9·18	0·042	9·17	0·040	9·17
Saanen	4·24	0·092	3·85	0·083	4·02	8·49	0·055	8·46	0·056	8·47
British Saanen	4·34	0·048	3·94	0·043	4·11	8·51	0·026	8·45	0·026	8·47
British	4·44	0·067	4·12	0·064	4·26	8·69	0·044	8·67	0·040	8·68
British Alpine	4·46	0·075	4·09	0·058	4·25	8·49	0·034	8·50	0·034	8·50
Toggenburg	3·82	0·060	3·81	0·076	3·81	8·42	0·046	8·38	0·046	8·40
British Toggenburg	4·77	0·078	4·29	0·096	4·50	8·87	0·050	8·85	0·052	8·86
English	5·41	0·159	5·27	0·148	5·33	9·22	0·069	9·08	0·055	9·14

As regards the solids-not-fat content, less variation is shown between breeds, and no difference between evening and morning samples is noted. The milk of the Anglo-Nubian is again significantly the richest. The British Toggenburg again takes second place, being significantly better than the remaining breeds with the exception of the British. The latter breed, however, is significantly better than the Saanen, British Saanen, British Alpine and Toggenburg between which the differences are not significant.

(*b*) *Other constituents.* Although the standard errors of the means of individual constituents of the milk are given in Table V, the small number of samples examined did not justify calculation of the standard errors of the differences. Conclusions have therefore only been drawn where the differences are so substantial as to make it obvious that they are real and not due to chance. These conclusions are as follows:

The lactose content of the milk is similar for most of the breeds but the values for the British breed tend to be low. So far as the milk proteins are concerned, the milk of the Anglo-Nubian is the richest, and that of the British Toggenburg is rather richer than that of the other breeds. The milk of the Saanen and British Saanen is low in ash, and this is mainly due to relative poorness in phosphoric acid. In contrast the milk of the Anglo-Nubian is high in phosphoric acid. As regards calcium, the milk of the Anglo-Nubian and the British Toggenburg is superior to that of the remaining breeds. The highest content of chloride occurs in the milk of the Saanen and the lowest in the milk of the Anglo-Nubian. The remaining breeds exhibit no differences in this respect.

Table V. *Mean percentages of constituents*

	Anglo-Nubian		Saanen		British Saanen		British		British Alpine		Toggenburg		British Toggenburg		English
	%	S.E.	%	S.E.	%	S.E.	%	S.E.	%	S.E.	%	S.E.	%	S.E.	%
Lactose	4·22	0·112	4·05	0·049	4·21	0·052	3·87	0·035	3·95	0·043	4·15	0·092	4·08	0·128	4·29
Nitrogen × 6·38	3·85	0·143	3·23	0·155	3·10	0·073	3·16	0·090	3·27	0·251	3·10	0·185	3·41	0·174	3·48
Total protein	3·46	0·145	2·79	0·151	2·76	0·069	2·63	0·065	2·77	0·198	2·74	0·181	2·92	0·157	3·12
Casein	2·91	0·171	2·39	0·190	2·34	0·052	2·30	0·062	2·39	0·164	2·26	0·087	2·55	0·114	2·54
Albumin + globulin	0·55	0·074	0·40	0·044	0·42	0·036	0·33	0·029	0·38	0·046	0·47	0·105	0·37	0·065	0·58
Non-protein nitrogen × 6·38	0·39	0·027	0·44	0·042	0·34	0·042	0·53	0·046	0·50	0·062	0·36	0·026	0·49	0·033	0·35
Ash	0·87	0·021	0·69	0·011	0·72	0·023	0·76	0·013	0·83	0·020	0·83	0·024	0·80	0·025	0·72
Calcium (CaO)	0·220	0·008	0·185	0·008	0·177	0·005	0·185	0·004	0·193	0·008	0·192	0·010	0·203	0·009	0·198
Chloride (Cl)	0·140	0·005	0·167	0·003	0·157	0·003	0·157	0·002	0·154	0·006	0·158	0·003	0·150	0·010	0·156
Phosphoric acid (P_2O_5)	0·317	0·017	0·220	0·022	0·237	0·010	0·261	0·018	0·271	0·022	0·285	0·017	0·289	0·021	0·274

Table VI. *Maximum and minimum values of the constituents of milk of breeds*

	Anglo-Nubian		Saanen		British Saanen		British		British Alpine		British Toggenburg		Toggenburg		English	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Fat	9·80	3·10	5·75	1·90	7·35	2·10	8·35	2·10	9·30	2·25	7·55	2·05	6·75	2·20	7·35	3·25
Solids-not-fat	10·95	7·15	9·34	7·30	10·08	6·91	10·54	7·65	10·36	7·06	10·54	7·30	10·28	7·12	10·53	7·80
Lactose	4·85	3·66	4·15	3·88	4·45	4·00	3·99	3·66	4·13	3·73	4·65	3·61	4·50	3·83	4·72	4·01
Ash	1·00	0·78	0·71	0·66	0·85	0·65	0·83	0·71	0·91	0·75	0·95	0·74	0·91	0·75	0·77	0·68
Calcium (CaO)	0·252	0·171	0·218	0·169	0·197	0·151	0·207	0·159	0·238	0·174	0·243	0·175	0·231	0·150	0·244	0·174
Chloride (Cl)	0·168	0·119	0·181	0·160	0·167	0·141	0·170	0·144	0·176	0·124	0·193	0·107	0·173	0·148	0·165	0·151
Phosphoric acid (P_2O_5)	0·421	0·245	0·314	0·181	0·28	0·194	0·343	0·136	0·362	0·146	0·360	0·246	0·346	0·227	0·281	0·267
Nitrogen × 6·38	4·46	2·95	3·74	3·00	3·52	2·90	3·43	2·67	4·34	2·70	4·06	2·93	3·85	2·68	3·57	3·38
Total protein	4·15	2·57	3·31	2·54	3·09	2·53	2·95	2·27	3·67	2·39	3·56	2·56	3·47	2·30	3·23	3·01
Casein	3·35	1·85	3·05	2·14	2·63	2·15	2·56	1·94	3·07	2·00	3·00	2·28	2·57	2·00	2·74	2·16
Albumin + globulin	0·91	0·25	0·49	0·26	0·58	0·30	0·46	0·14	0·60	0·22	0·72	0·18	0·90	0·30	0·97	0·29
Non-protein nitrogen × 6·38	0·55	0·31	0·53	0·33	0·52	0·20	0·86	0·37	0·72	0·31	0·66	0·37	0·42	0·28	0·47	0·25

Table VII. *Percentage of samples from breeds falling within specified limits of fat and solids-not-fat content*

Range of percentage	Anglo-Nubian	Saanen	British Saanen	British	British Alpine	British Toggenburg	Toggenburg	English
				Morning fat				
2– 3	—	—	12	10	9	4	8	—
3– 4	6	64	42	38	43	38	65	6
4– 5	32	32	37	36	34	31	21	40
5– 6	34	4	8	13	10	20	3	29
6– 7	20	—	1	3	3	5	3	19
7– 8	5	—	—	—	1	2	—	6
8– 9	2	—	—	—	—	—	—	—
9–10	1	—	—	—	—	—	—	—
				Evening fat				
2– 3	—	4	5	4	2	—	7	—
3– 4	2	38	33	33	39	20	61	12
4– 5	19	42	41	37	39	42	28	29
5– 6	31	16	15	20	11	31	3	27
6– 7	33	—	5	5	5	5	1	24
7– 8	10	—	1	1	3	2	—	8
8– 9	2	—	—	1	1	—	—	—
9–10	3	—	—	—	—	—	—	—
				Morning solids-not-fat				
7– 8	2	12	17	5	17	5	19	—
8– 9	37	82	72	64	66	60	74	42
9–10	50	6	11	29	16	30	5	58
10–11	11	—	—	2	1	5	2	—
				Evening solids-not-fat				
7– 8	2	8	12	7	13	—	10	2
8– 9	38	79	75	67	72	64	80	31
9–10	49	13	12	21	14	31	10	60
10–11	11	—	1	5	1	5	—	7

THE EFFECT OF STAGE OF LACTATION ON YIELD AND COMPOSITION OF THE MILK

From the yields of milk and dates of kidding supplied with the samples, the curves given in Figs. 5–7 have been drawn. These curves are based upon the following numbers of samples, which consisted of evening and morning milk of the same day. The figures plotted refer to the milk of the whole day:

British Saanen	...	...	424
Other breeds	...	...	1156
All breeds ...	...	...	1580

The period covered by the curves is representative for the majority of goats, but, as previously stated, the lactation period of some animals may extend over a number of years. Such animals have been excluded. The first value covers a period of from 5 to 20 days after kidding, by which time the secretion has lost its colostral character(8).

The yield curve of the breeds, excluding British Saanen, is similar to that of cows. The yield of the British Saanen declines much more slowly with the advancement of lactation than the yield of other breeds, especially between the second and fifth months, and with many animals a high yield is maintained

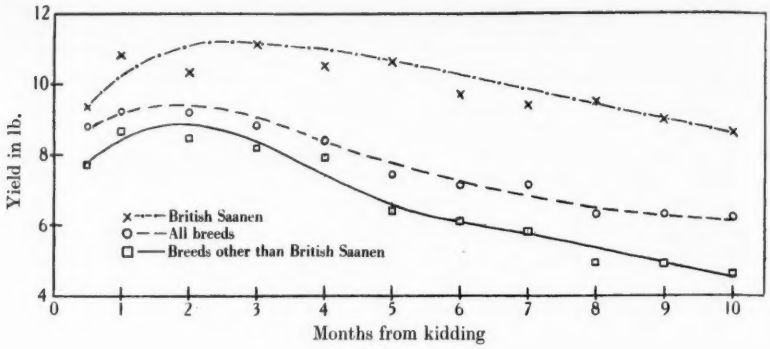

Fig. 5.

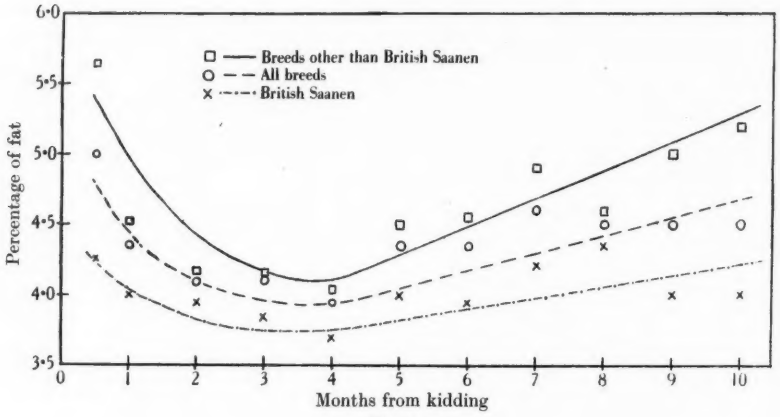

Fig. 6.

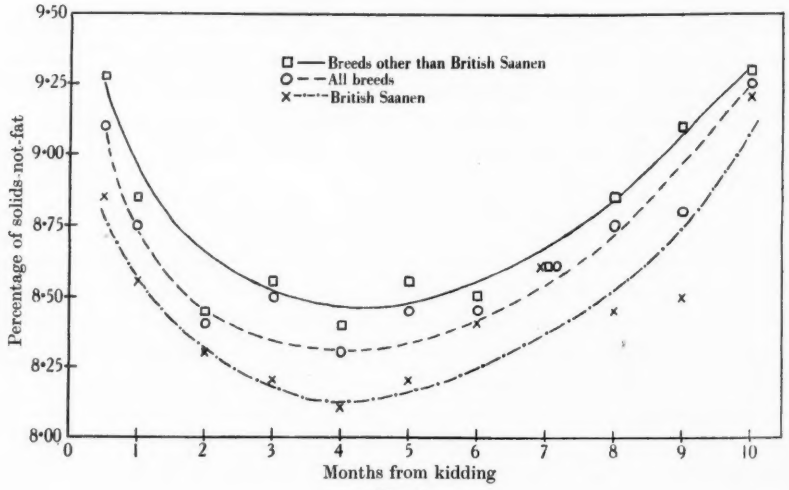

Fig. 7.

until the onset of the next kidding. With all breeds, however, the maximum yield is attained in the first month and little falling off is shown until the end of the fourth month.

The fat percentages in the milk of all breeds fall to a minimum in the fourth month, rise during the ensuing 3 months and thereafter remain practically constant. The milk of the cow, on the other hand, usually shows a marked rise in fat percentage during the last 2 or 3 months of lactation.

The decline and rise in percentages of solids-not-fat are similar in the milk of the goat and the cow, minimum values occurring at about the fourth month.

The average daily yields of fat and solids-not-fat of all goats show the same general trend as the daily yields of milk, the maximum values being reached at 2 months from kidding; from this point a gradual decrease occurs until the end of the lactation. The maximum and minimum values noted were respectively 0·42 and 0·27 lb. fat and 0·87 and 0·53 lb. solids-not-fat.

EFFECT OF AGE OF GOAT ON YIELD AND COMPOSITION

Owing to the inadequacy of the available data consideration of the effect of age on the yield and composition of goat's milk has had to be confined to animals between the ages of 2 and 4 years at commencement of their lactation. Such animals furnished about 80% of the samples examined. The relevant figures are as follows:

Age in years	Average yield in 300 days lb.	% fat	% solids-not-fat
2	2220	4·43	8·69
3	2640	4·42	8·61
4	2850	4·45	8·58

It may be noted that the average yield rises until 4–5 years of age is reached, and that the composition of the milk is almost constant during that time. From the data from the much smaller number of animals exceeding this age, there are indications that the yield falls with increasing age from about 5 years.

THE MILK YIELDS OF THE BREEDS

The milk yields of each of the breeds have been worked out for officially recorded lactations of not less than 250 days for the years 1926–36. The mean yields are given in Table VIII, together with the weights of fat and solids-not-fat represented by these yields, as calculated from the mean percentages of these constituents which have been found in the present investigation. It must, however, be borne in mind that, as these figures do not include the large number of animals not officially recorded (which probably give lower yields), it is likely that the average yield of all goats in the country is about 30% less than that recorded in Table VIII.

Table VIII. *The yields of the breeds*

Breed	Number of lactations	Average yield per lactation in lb.		
		Milk	Fat	Solids-not-fat
British Saanen	172	2917	123	247
Saanen	63	2614	105	221
British Alpine	117	2500	106	212
British Toggenburg	43	2393	108	212
British	947	2143	91	186
Toggenburg	72	2040	78	171
Anglo-Nubian	64	1846	103	169
English	51	1424	—	—

From the above table it is apparent that the average yield of milk from all officially recorded goats in Great Britain is about 7½ lb. per day. Moreover, a study of officially published milk records shows that steady progress is being made, and there is no reason to believe that the present record lactation yield of 5480 lb. represents finality. In this connexion it may be noted that there is relatively less disparity between average and record yields with the goat than with the cow.

Summary

An account has been given of observations made for over two years on the composition of the milk of the breeds of goat distributed throughout Great Britain. The average percentages of fat and solids-not-fat in the milk of the breeds, together with their standard errors, are recorded. The frequency with which evening and morning samples fell within specified limits of these constituents is indicated for each breed, and diagrammatically for all goats. Detailed analyses of the milk of each breed are given, with the maximum and minimum values encountered and the standard errors of the average figures. Comparison is made of the composition of the milk of the goat with that of the cow. The effect of stage of lactation on yield of milk and on percentages of fat and solids-not-fat are shown by means of curves. The effect of age on the yield and composition of milk is indicated. The average yields per lactation for each breed, based upon the records of animals officially recorded during the last ten years are noted.

Acknowledgements

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185. THE INCIDENCE OF MASTITIS IN COWS YIELDING MILK LOW IN SOLIDS-NOT-FAT

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From time to time references to the occurrence of milk falling below the legal minimum of 8·5% of solids-not-fat appear in the literature. It is well known that such milk is not limited to cows showing clinical evidence of disease, but may also be produced by apparently healthy animals. Various reasons, such as feeding, seasonal changes and weather conditions, have been advanced to account for the deficiency. In a few cases attempts have been made to correlate the presence of streptococcal mastitis with changes in the chemical composition of the milk.

Dahlberg *et al.* (1) examined two herds, one Jersey and one Friesian, and dealt only with milk of normal macroscopic appearance from cows showing no clinical evidence of disease of the udder. The cows were divided into three groups on the basis of a bacteriological test: (*a*) showed no demonstrable infection, (*b*) slight infection, and (*c*) a pronounced latent infection, but the milk was normal in appearance. The writers deduce from their results that the chemical composition of *mixed* milk, of normal macroscopic appearance, produced by the two herds showing evidence of latent mastitis, did not deviate significantly from the normal, although among other things there was a tendency for the "skim-milk solids" to decrease by as much as 0·67% in individual quarter samples.

Peskett & Folley (2), in examining cow's milk low in solids-not-fat, suggested that at least in some cases the decrease might be due to dilution of the normal udder secretion by a fluid of the nature of oedema fluid. W. L. Davies (3) suggested that milk low in non-fatty solids resulted from the infiltration of an isotonic diluent similar to lymph serum. He discussed the possibility that the cause of the secretion of the diluent was a chronic catarrhal condition of the udder following an attack of mastitis.

Rowland (4), examining the protein distribution in normal and abnormal milk of a comparatively few cows, concluded that the milk from cows with clinical mastitis was deficient in solids-not-fat. A number of apparently healthy cows known to have produced milk low in solids-not-fat for some time were, with two exceptions, shown to be suffering from subclinical mastitis when examined by bacteriological methods. Further work by Rowland & Zein-El-Dine (5) has confirmed this conclusion.

It is well recognized that mastitis is probably the most prevalent and widely distributed disease of dairy cows. It is somewhat difficult to estimate the

incidence in this country, as various workers give widely different figures. S. J. Edwards (6), writing of "chronic" or "streptococcal mastitis", stated that about 30% of milking cows were affected, although many appeared normal. In our experience the percentage of infected cows in different herds varies considerably and in many herds may be very high.

SOURCE OF DATA

Material for a study of the interrelation of mastitis and milk low in solids-not-fat was provided by a field investigation carried out from this Institute during the winter of 1936–7. The herds were situated in the counties of Berks, Dorset, Hants, Oxon and Somerset.

In this investigation twenty-nine herds, comprising over the whole period 934 cows, were used primarily for the study of the protein requirements of dairy cows. During the experimental period all the cows in milk received controlled rations. In general, however, their management was that of average or good dairy farms in the south of England, and the rations approximated closely to the normal. The breeds were represented as follows: sixteen Shorthorn herds, three Friesian herds, four Guernsey herds, three Jersey herds, one Ayrshire herd, two herds of various breeds.

Throughout the winter the milks of all cows in milk were sampled by the milk recorders and sent to the Institute, usually at 5- or 6-weekly intervals. Morning and evening samples were taken separately from each cow. The fat content was estimated by the Gerber method, and the solids-not-fat content calculated from the density and fat percentage.

With the results of periodic examination of the solids-not-fat content of the milk of individual cows at our disposal, it was possible to visit each of the twenty-nine herds at least twice during the first three months of 1937, and collect information on the incidence of mastitis among those cows whose milk was definitely below the standard in solids-not-fat.

TECHNIQUE

(*a*) *In the field*

In normal practice the farmer has no satisfactory means of recognizing subclinical cases of mastitis, and it is not unusual for mild clinical cases to be overlooked. In our study of the group of cows classed in the "low solids-not-fat" category, the information which was collected on the farm was therefore limited to advanced clinical cases, in which at some time in the milking history of the animal, one or more quarters, or the milk therefrom, was obviously abnormal to the milker concerned.

Before visiting each herd the solids-not-fat records of each milking cow at the last visit of the milk recorder were studied, and the names of "low solids-not-fat" cows listed. For this purpose only those animals which gave milk with less than 8·5% solids-not-fat at both evening and morning milkings were

included in our examination. Further, we did not concern ourselves with animals which had been in milk for more than 8 months, or were giving less than 10 lb. milk daily. It was considered that the mere fact that an animal was stale might materially influence the chemical constitution of its milk. On the farm the herdsman concerned was asked if any listed animal was at that time suffering from mastitis or had previously shown clinical evidence of the disease. If the answer, for any animal, was in the affirmative, the cow was considered to be positive and the case followed no further. Usually, however, the "low solids-not-fat" cow was not known to have had clinical evidence of the disease or the occurrence had been forgotten. This means that clinical mastitis for the purposes of this paper was a condition which could be recognized by the skilled cowman in intimate contact with his animals. Similarly, "subclinical" mastitis means a condition not so recognizable. In these cases the freshly drawn milk was tested with brom-cresol papers and a sample brought to the laboratory for bacteriological examination. In taking these samples the milk immediately following the foremilk was milked directly into a sterile bottle. Approximately equal quantities from each quarter were included in all samples. In no instances were single-quarter samples examined. In a few cases pronounced alkaline reaction to brom-cresol papers was accepted as indication of mastitis, but poor correlation between the reaction to brom-cresol and the result of the bacteriological test discouraged the use of the former method as a final criterion of the presence or absence of the disease. It is well recognized, of course, that milks which are negative to the brom-cresol test are not necessarily free from mastitis infection. Further, in several instances milks which were negative to the bacteriological test had been recorded as giving doubtful reactions to the brom-cresol test. On two occasions milk from cows which were not stale, and recorded as positive to the brom-cresol test, showed no bacteriological evidence of mastitis. In view of these facts only eight cows in all in the early stages of the investigation were regarded, on the result of the brom-cresol test alone, as suffering from mastitis.

(*b*) *Bacteriological examination*

Samples were examined within 24 hr. of milking for the presence of streptococci. 10 ml. quantities were centrifuged at 2500 rev./min. for 5 min. Two (or in cases with heavy deposits three) dilutions of the resulting deposit were plated on Edward's[7] crystal violet aesculin blood agar. When very heavy deposits were obtained a portion was also inoculated into veal infusion broth *p*H 7·4 and examined for the presence of streptococci. After incubation at 37° C. for 24 hr. the plates were examined. Those showing large numbers of haemolytic colonies characteristic of *Str. agalactiae* were at once recorded as positive. Where only a few haemolytic colonies, or non-haemolytic non-aesculin-splitting colonies were present, representative colonies were picked off into dextrose lemco broth, *p*H 7·4. The resulting cultures were examined

microscopically, and all consisting of streptococci were centrifuged and the deposits extracted with $N/20$ HCl according to Lancefield's technique(8). Precipitin tests were carried out against antisera for mastitis streptococci of Stableforth's Groups I and II(9). Where doubtful or negative results were obtained, reactions in litmus milk, methylene-blue milk, salicin, mannitol, inulin, aesculin, sodium hippurate broth and heat resistance tests (Minett(10)) were carried out.

Care was taken not to confuse haemolytic colonies of *Str. agalactiae* with other organisms which might give zones of haemolysis. On several occasions streptococci of the *Str. infrequens* type were encountered, and in two milk samples, at least, these organisms were present in large numbers.

All the cases of mastitis reported here, on the grounds of bacteriological tests were, with one exception, found to be due to *Str. agalactiae* (Stableforth, Group I). The remaining case was caused by an organism giving rise to an acute form of mastitis and falling into Stableforth's Group II.

Results

The results are set out in Tables I and II. The numbers of herds representing each breed were insufficient for an interbreed comparison. We consider, however, that since the proportions of each breed represented are roughly equal to those of the various breeds in the south of England, our results, *en masse*, represented approximately the position in that district during the first three or four months of 1937.

Table I. *Number and percentage of cows yielding milk low in solids-not-fat in twenty-nine herds*

	Shorthorn	Friesian	Jersey and Guernsey	Other herds	All herds
			First examination		
Number of cows sampled for solids-not-fat prior to examination for mastitis	481	110	112	69	772
Number of cows which gave milk low in solids-not-fat at two consecutive milkings	64	59	8	23	154
Percentage of cows which gave milk low in solids-not-fat at two consecutive milkings	13·3	53·6	7·1	33·3	19·9
			Second examination		
Number of cows sampled for solids-not-fat prior to examination for mastitis	492	107	113	75	787
Number of cows which gave milk low in solids-not-fat at two consecutive milkings	65	56	9	20	150
Percentage of cows which gave milk low in solids-not-fat at two consecutive milkings	13·2	52·3	8·0	26·7	19·1

Table I shows that on first examination of samples from 772 cows, 154 or 19·9% were below 8·5% solids-not-fat at two consecutive milkings. In our second examination, in a population of 787 cows, 150 or 19·1% failed to reach

the standard. In the first examination thirty-nine, and in the second examination thirty-four cows were stale, and our investigations were directed to the mastitis history of the remaining 115 and 116 cases respectively. Since our data were assembled after two visits to each farm, it often happened that a cow giving low solids-not-fat on one occasion did so again on the second occasion. In tabulating our results we have, for the sake of brevity, taken each visit as if to a separate farm, and the incidence of low solids-not-fat refers to one specific occasion (but two consecutive milkings) without reference to previous tests.

Table II. *Incidence of mastitis in cows which yielded milk low in solids-not-fat and which were not "stale"*

	Shorthorn	Friesian	Jersey and Guernsey	Other herds	All herds	
					No.	%
		First examination				
Number of low "solids-not-fat" cows examined	51	45	4	15	115	—
Cows having definite clinical evidence of mastitis	4	12	1	4	21	18·3
Cows assumed to have mastitis (strong brom-cresol reaction)	3	0	1	4	8	7·0
Cows positive to bacteriological test	16	13	0	5	34	29·6
Total number of cows infected	23	25	2	13	63	54·8
Cows negative to bacteriological test	20	20	2	2	44	38·3
Cows not completely examined	8	0	0	0	8	7·0
		Second examination				
Number of low "solids-not-fat" cows examined	50	42	6	18	116	—
Cows having definite clinical evidence of mastitis	6	8	2	6	22	19·0
Cows assumed to have mastitis (strong brom-cresol reaction)	0	0	0	0	0	0
Cows positive to bacteriological test	22	23	3	9	57	49·1
Total number of cows infected	28	31	5	15	79	68·1
Cows negative to bacteriological test	22	11	0	3	36	31·0
Cows not completely examined	0	0	1	0	1	0·9

Table II shows the summarized results of our first and second field and laboratory examinations of the low "solids-not-fat" cows. In the first examination evidence of mastitis was found in 54·8% and in the second examination 68·1% of the low "solids-not-fat" cows. By combining all the results shown in Table II, it is found that whereas the owners or herdsmen could tell us of only 18·6% of the cases, bacteriological examination revealed a further 39·4% of infection. In other words there were about twice as many subclinical cases of mastitis as clinical.

Our records show that of the forty-four cows that were negative to the bacteriological test in the first examination, twenty cases were retested in the second examination, which took place after a period varying from 5 to 8 weeks. Fourteen of these were again returned as negative while six were then positive. It should not be assumed that the first test failed to reveal the presence of the disease in these six animals. Three of them were in herds where the disease was rampant and may well have been infected during the intervening period.

Solids-not-fat in "mastitis" cows

It will be realized that we have limited the scope of our investigation to cows yielding milk low in solids-not-fat. It would therefore be very interesting to know the proportion of cows suffering from mastitis which yield milk below the legal standard for solids-not-fat. Preliminary data on this subject are available from only one herd, which has been tested bacteriologically for the presence of mastitis, irrespective of the quality of the milk yielded by each animal. In this large herd information has so far been collected concerning the chemical quality of the milks of individual cows on three occasions and concerning the incidence of mastitis on two occasions. Thirteen animals were positive to the bacteriological test in November 1937, and the same cows were positive again in February 1938. Table III shows the range in the solids-not-fat content of the individual milks of these animals. When evening and morning samples were tested in October 1937 and January and February 1938, two cows only were consistently above and four consistently below 8·5%. The remainder fluctuated about the minimum standard.

Table III

Cow no.	Range in percentages of solids-not-fat in 3 morning milkings and 3 evening milkings, 27. x. 37, 6. i. 38, 24. ii. 38
1	8·8–9·1
2	8·7–9·4
3	8·5–9·1
4	8·4–9·2
5	8·4–8·7
6	8·3–8·8
7	8·3–8·7
8	8·3–8·6
9	8·2–8·5
10	8·0–8·4
11	8·0–8·4
12	7·7–8·3
13	7·5–8·4

Discussion

The results of our two examinations provide definite evidence that at least 60% of animals which were giving milk low in solids-not-fat and which were not stale were also suffering from mastitis infection. There are two good reasons for suspecting that the estimated incidence of infection is in fact low. (1) When bacteriological tests on animals which had been reported as negative in the

first test, carried out 5–8 weeks earlier, were repeated, a considerable number were found to be infected. It may therefore be suspected that an occasional "positive" was missed at the first test. It is generally recognized that a proportion of cows excrete the organism intermittently, and our repeat samples support this view. (2) It must be remembered that we were not dealing with single-quarter samples. The bulking of the four quarters would obviously cause dilution of the organisms accompanying a mild infection in one quarter.

In general, therefore, we feel that the technique used may well have failed to reveal a substantial number of cases of mastitis, and having in mind the facts presented earlier (p. 170) it may be estimated that the total mastitis infection in our low "solids-not-fat" cows amounted to about 70%.

The high percentage of mastitis infection found in this field investigation is of the order of that reported by Rowland & Zein-El-Dine (5). The conditions of their comparisons were more stringent than ours, and this probably accounts for the higher percentage of infection reported by them. It should be pointed out that Rowland & Zein-El-Dine's clinical and bacteriological tests were carried out on the same samples, whereas ours were taken at different times; this, and the fact that these workers used individual quarter samples, would obviously result in a higher correlation between low solids-not-fat and mastitis infection.

Nevertheless, our results are sufficiently definite to show that where difficulty with milk low in solids-not-fat is experienced one of the first steps should be the control and eradication of clinical and subclinical mastitis. It is obviously useless to attempt to improve the chemical quality of the milk by nutritional means alone when unsuspected mastitis infection can produce so pronounced an effect.

Summary

1. In a field investigation covering twenty-nine herds, an average of 19·5% of the animals in milk during the first three months of 1937 were yielding milk low in solids-not-fat, estimated by the routine method, at two consecutive milkings.

2. Of the low solids-not-fat cows which were not stale, evidence of mastitis infection was found in an average of 61·5% of cases. Re-examination of some negative animals suggests that the total infection amounted to about 70%.

3. There were apparently at least twice as many "subclinical" cases of mastitis as clinical cases.

We are indebted to Dr A. T. R. Mattick and Mr J. Mackintosh for their advice and interest in this work, and to Mr A. Wagstaff for the solids-not-fat determinations.

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186. THE CASEIN NUMBER. A CHEMICAL METHOD FOR DIAGNOSIS OF MASTITIS

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(With 1 Figure)

Control of the streptococcal mastitis which is so prevalent in dairy herds necessarily depends on accurate diagnosis, and much attention has been devoted to comparisons of the various methods for the detection of latent subclinical infection (1,2,3,4). Direct bacteriological methods aim at demonstrating the causal organism and are reliable, but the cultural examination of samples of milk on selective media for *Str. agalactiae*, and the verification of doubtful colonies, demand much time and skill as well as special facilities. Moreover, a small proportion of infected quarters does not give positive results at every test, and several samples of milk may have to be examined.

Indirect chemical methods aim at revealing changes in the composition of the milk as a result of the infection. Tests for the cell, catalase and chloride contents of the milk, and its reaction to indicators, are used. Although rapid and simple, they are not reliable, and are of value only as auxiliary tests. They give negative results for an appreciable percentage of infected animals, and positive results for healthy animals approaching the end of lactation (3,4).

A reliable method of diagnosis that could be carried out quickly in a chemical laboratory would be of considerable value. In an earlier communication, which recorded data for the full protein nitrogen distribution in samples of milk from normal cows and cows with clinical and subclinical mastitis, Rowland (5) suggested that mastitis could be detected by the casein nitrogen/total nitrogen ratio in the milk; and this was conveniently expressed as the $\frac{\text{percentage of casein N}}{\text{percentage of total N}} \times 100$, and called the "casein number". The use of the casein number as a method of diagnosis has now been examined under strict bacteriological control, and the full results are detailed below.

Experimental

Cultural and chemical examinations have been made of samples of milk from 247 quarters of sixty-two cows of the Shorthorn, Friesian, Ayrshire and Guernsey breeds. The cows belonged to four herds which were almost free from clinical cases of mastitis. Of the 247 quarters, only eight showed clinical signs

on palpation, so that the reliability of the casein number has been tested on the subclinical cases of mastitis which are often not detected by the chemical methods commonly employed.

Sampling

The first streams of milk from each washed quarter were drawn into a black dish, carefully inspected for the presence of clots, and discarded. Then about 20 ml. of milk were drawn into a sterile tube for the bacteriological examination, followed by 2–4 oz. into a bottle for the chemical examination. A few clots, mostly very small, were found in the foremilk from eighteen quarters, but the samples actually collected were of normal appearance. The bacteriological and chemical examinations of most of the samples were commenced within a few hours. The remainder were stored overnight at a low temperature before being tested.

Bacteriological examination

The centrifuge deposit from 10 ml. of milk was plated on the selective crystal violet aesculin blood agar medium of Edwards (6) and examined for the presence of *Str. agalactiae*. This work, which requires considerable experience, was carried out entirely by one of us (M. Z.).

Chemical examination

The total nitrogen and casein nitrogen contents of the milk were determined by the rapid semi-micro Kjeldahl method described fully in a previous paper (7); the 10 ml. portions of milk taken with the same pipette for both total nitrogen and casein nitrogen were not weighed. It is essential that this method of precipitation should be used, since other procedures do not cause complete precipitation, and if followed would yield casein numbers not comparable with those discussed here.

Advantage was taken of the bacteriological control to investigate the effect of subclinical mastitis on the solids-not-fat content of milk, and for this purpose the total solids content of the samples was determined by evaporation and the fat content by the Gerber method. The data for solids-not-fat are considered in a subsequent paper (8).

Some of the quarters were sampled more than once, at intervals of about a week, to ascertain if there were changes in either the cultural or the chemical findings. As was expected, a few infected quarters failed to give positive cultural results at every test, but the chemical data showed no significant change except in the samples from cows Nos. 44 and 58, and for these cows the two sets of figures are given in full (Table III). The second sets were used in the final calculation of results, so that each of the 247 quarters was included once only in the summary of averages.

Results

The incidence of infection for the cows and individual quarters of each breed is shown in Table I. Of the sixty-two cows examined, 74% were found to be infected in one or more quarters, and of the 247 quarters examined 49% were found to be infected, and 5% considered doubtful.

Table I. *The incidence of infection*

	Cows			Quarters		
Breed of cow	Total no.	No. infected	% of total infected	Total no.	No. infected	% of total infected
Shorthorn	25	19	76	99	44	44
Friesian	26	20	77	104	57	55
Ayrshire	7	6	86	28	19	68
Guernsey	4	1	25	16	1	6
All breeds	62	46	74	247	121	49

The total nitrogen content of the samples varied from 362·0 to 687·4, and the casein nitrogen from 153·0 to 554·0 mg. per 100 ml. The casein number varied from 42·3 to 83·4, and the solids-not-fat in the fat-free milk from 4·26 to 10·28%.

The total nitrogen and casein nitrogen contents and the casein numbers are summarized in Table II. The total nitrogen in the milk from the uninfected quarters of the Shorthorn, Friesian, Ayrshire and Guernsey cows averaged 542, 531, 590 and 594 mg. per 100 ml. respectively, and the corresponding infected quarters 544, 478, 528 and 621 (one sample) mg. per 100 ml.

The casein nitrogen in the milk from the uninfected quarters of the Shorthorn, Friesian, Ayrshire and Guernsey cows averaged 429, 427, 469 and 477 mg. per 100 ml. respectively, and from the corresponding infected quarters 390, 354, 371 and 486 (one sample) mg. per 100 ml.

The mean casein numbers for the uninfected samples of each breed showed little variation at 79·2, 80·4, 79·5 and 80·3, but for the infected samples they were much lower and very variable.

Table II. *Averages for total nitrogen and casein nitrogen contents and casein numbers*

Breed of cow	No. of quarter samples	Average total nitrogen (mg. per 100 ml. of milk)	Average casein nitrogen (mg. per 100 ml. of milk)	Casein no. Mean	Casein no. S.D.
		Samples not infected			
Shorthorn	50	542	429	79·2	1·1
Friesian	43	531	427	80·4	1·4
Ayrshire	7	590	469	79·5	0·7
Guernsey	14	594	477	80·3	1·1
		Samples infected			
Shorthorn	44	544	390	71·7	6·9
Friesian	57	478	354	74·1	6·4
Ayrshire	19	528	371	70·3	5·7
Guernsey	1	621	486	78·3	—

The reliability of the casein number

The total nitrogen of milk consists of casein, albumin, globulin, proteose-peptone and non-protein nitrogen, and the value of the casein number for the detection of mastitis is based on two observations(5), which are amply borne out by the figures in Table II, i.e. (1) that in the milk secreted by healthy quarters, although the content of total nitrogen may vary over a wide range, the casein accounts for a virtually constant percentage of that total nitrogen—a uniformity in the composition of a biological product which is surprising; and (2) that in the milk secreted by infected quarters the casein nitrogen accounts for a smaller percentage of the total nitrogen than in normal milk, as there is a decrease in the casein content and increases in the albumin, globulin, and proteose-peptone contents.

If, therefore, it can be shown that the casein number of normal milk is sufficiently constant to be used as a standard for such milk, a lower casein number will characterize an infected sample, and the extent of the lowering of the number will give a measure of the extent to which the presence of infection is disturbing the animal's secretory mechanism.

Fig. 1, where the full bacteriological results, casein numbers, and solids-not-fat percentages are recorded graphically, shows clearly the lower casein numbers of the infected samples, and the very short range covered by the casein numbers of normal samples, the division between infected and normal samples falling at a value for the casein number of 78·0.

If, for the diagnosis of mastitis by the casein number, a figure above 78·0 be taken as indicating a normal sample, and a figure of 78·0 and below an infected sample, the verdict of the chemical as compared with the bacteriological method is as follows:

Bacteriological result	Casein number result	Number of samples
Negative	Negative	106
Positive	Positive	116
Negative	Positive	9
Positive	Negative	12

Thus 128 and 125 samples are reported positive by the bacteriological and chemical tests respectively, and 115 and 118 negative. Of the 128 samples found positive bacteriologically, 116, i.e. 90·6%, are positive chemically. Of the 115 samples found negative bacteriologically, 106, i.e. 92·2%, are negative chemically. The proportion of instances in which there is disagreement between the two tests is 8·6%, i.e. 21 out of the 243 samples. Some of these discrepancies may not be real. Of the nine quarters reported negative bacteriologically and positive chemically four have abnormally low casein numbers, i.e. 75·1, 76·4, 76·8 and 76·8. It is possible that in these instances the bacteriological tests were at fault. This might be explained on two grounds. Firstly, these quarters were sampled only once, and it is possible that if further samples had been taken the bacteriological examination would have been positive. In this connexion it may be mentioned that certain quarters which were examined

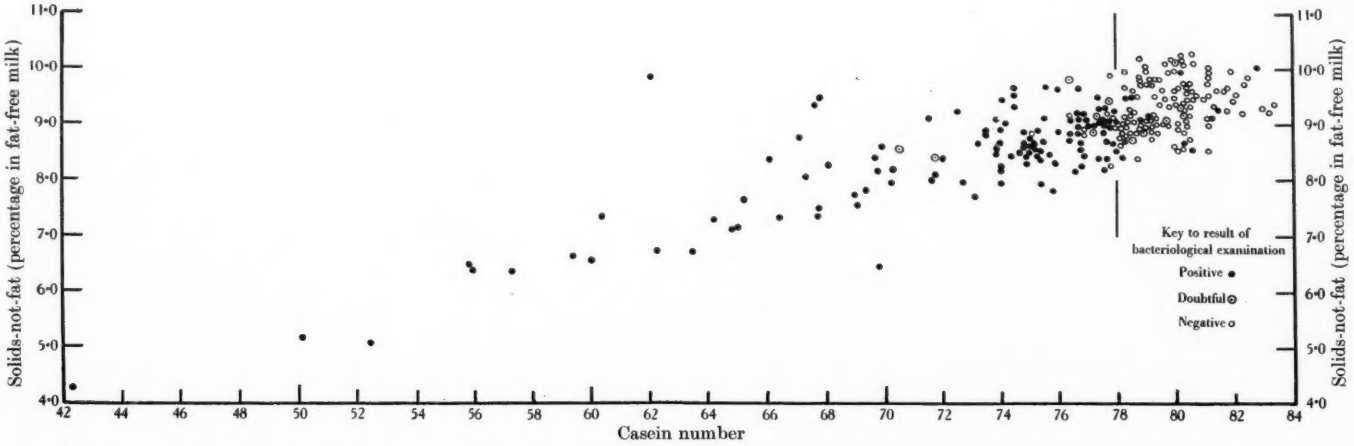

Fig. 1. The relationship between the casein number, the bacteriological examination, and the solids-not-fat percentage.

bacteriologically more than once did not give positive results regularly. Secondly, though less likely, these quarters may have been infected, not with *Str. agalactiae*, but with organisms of other types such as staphylococci. Such infections, although much less common, are by no means rare.

For the remaining five quarters which were reported negative bacteriologically and positive chemically the casein numbers (77·8–78·0) are close to the dividing line of 78·0, as are the numbers (78·2–78·3) for three of the quarters reported positive bacteriologically and negative chemically. This slight overlap of positive and negative results at the dividing line fortunately affects a very small proportion of the total quarters. It suggests that for a casein number of 78·0 the bacteriological condition is best reported as "doubtful", and the quarter sampled again. This procedure is sometimes necessary with the bacteriological test.

Nine quarters are reported positive bacteriologically which have casein numbers of 78·8–82·8, i.e. much above the dividing line. An indication of their nature is provided by the two sets of results for cow No. 44. The three highest of these unexpected figures were obtained at the first sampling of the cow, and, as the data of Table III 44*a* and *b* show, within 9 days changes took place in the composition of its milk, and the casein numbers of the three

Table III. *Changes in the composition of infected milk*

Quarters are listed in the order right fore, right hind, left fore, left hind.

Cow number and breed	Bacterio-logical examination	Solids-not-fat % in fat-free milk	Total N mg. per 100 ml.	Casein N mg. per 100 ml.	Casein number
Friesian No. 44*a*	+	9·31	503·0	409·3	81·4
24 January 1938	+	9·14	507·6	412·1	81·2
	+	10·03	501·9	415·1	82·8
	+	8·27	507·0	379·9	74·9
Friesian No. 44*b*	+	8·18	541·7	420·3	77·6
2 February 1938	+	5·14	390·0	195·5	50·1
	+	8·18	446·2	313·7	70·3
	+	8·26	506·9	344·8	68·1
Ayrshire No. 58*a*	–	9·04	516·6	404·5	78·3
30 June 1937	+	7·80	530·7	401·9	75·8
	+	9·48	525·4	406·6	77·4
	+	8·86	530·6	409·6	77·2
Ayrshire No. 58*b*	+	7·91	463·1	324·9	70·2
8 July 1937	+	6·72	481·1	305·4	63·5
	+	7·52	473·2	327·1	69·1
	+	7·33	472·9	314·1	66·4

quarters dropped from 81·4, 81·2 and 82·8 to 77·6, 50·1 and 70·3. It is possible that at the time of the first sampling the quarters were in the very early stage of infection when the bacteria had not yet reached actual milk secreting tissue, being confined, for example, to the milk cistern. Cow No. 58 also illustrates well the rapidity with which the composition and casein number of the milk of an infected quarter may change.

Hence, when a large number of quarters is examined by the casein number for the presence of infection, a few may be in the initial stages of infection and not detected by a single sampling, but this would be detected by sampling

again at a later date. In this respect the chemical method of diagnosis is subject to the same criticism as the bacteriological method, which may also occasionally fail to detect some mildly infected quarters by a single sampling.

The above remarks show that to have made it possible to assess definitely the relative reliabilities of the bacteriological and chemical methods it would have been necessary to test statistically the results obtained by examining a large number of quarters several times by each method. An investigation on such a scale was not possible. It is probable, however, that the figure of 8·6% given above for the error involved in the chemical method is too high and that in practice the casein number would provide a valuable and reliable method for the diagnosis of mastitis.

Advantages of the casein number

The casein number method of diagnosis makes it possible for the chemist to participate with greater accuracy than hitherto in the detection that is the essential preliminary to the control and eradication of mastitis. The method has an advantage in that the sample of milk may be drawn without special precautions into an unsterilized bottle, and in that the determination and interpretation of the number do not present any difficulty in the ordinary chemical laboratory in the form of either experience or materials. Moreover, a result may rapidly be obtained (2½–3 hr.). The method has the further advantage that it provides a measure of the extent to which the infection is disturbing the secretory function of the mammary gland.

Summary

The accuracy of the casein number $\left(\frac{\text{percentage of casein N}}{\text{percentage of total N}} \times 100\right)$ of milk as the basis for a chemical method for the detection of mastitis has been investigated. The number was determined for 247 samples of milk from the individual quarters of sixty-two cows, and the samples were examined bacteriologically for mastitis streptococci. Of the 247 quarters 49% were found to be infected—mostly subclinically—involving 74% of the cows.

The total nitrogen content of the samples varied from 362·0 to 687·4 mg. per 100 ml., and the casein nitrogen from 153·0 to 554·0. The casein number varied from 42·3 to 83·4. The casein number averaged 79·2, 80·4, 79·5 and 80·3 in the samples from the uninfected quarters of the Shorthorn, Friesian, Ayrshire and Guernsey cows respectively; and 71·7, 74·1 and 70·3 in the samples from the infected quarters of the Shorthorn, Friesian and Ayrshire breeds respectively.

For the diagnosis of mastitis from the casein number, a figure of 78·0 and less was taken as indicating an infected quarter, and on comparing this chemical method with the bacteriological method, out of a total of 243 quarters, the chemical result differed from the bacteriological for 21 quarters—representing 8·6% of the total.

Reasons are given for considering that this estimate of the error is excessive and that in practice the casein number would provide a valuable and reliable method for the diagnosis of mastitis.

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187. THE EFFECT OF SUBCLINICAL MASTITIS ON THE SOLIDS-NOT-FAT CONTENT OF MILK

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In a previous paper the widespread occurrence of milk samples with a low content of solids-not-fat was discussed, and milk samples of normal appearance, produced by apparently healthy cows and having persistently a low content of solids-not-fat throughout lactation, were shown on bacteriological and chemical evidence to be of two kinds: (*a*) samples from cows with latent subclinical mastitis, i.e. pathological samples, and (*b*) samples from uninfected cows, the cause of the low solids-not-fat being physiological. The total number of samples examined was too small for any definite statement to be made as to the relative occurrence of these two kinds, but it was observed that a high percentage of the cases of low solids-not-fat was probably due to mild subclinical mastitis(1).

In subsequent work milk samples were collected from the individual quarters of 62 cows during the course of an investigation of the casein number as an index of subclinical mastitis(2), and the opportunity was taken to investigate further the effect of the subclinical mastitis on the solids-not-fat content of the milk. Samples from individual quarters reveal the effect more clearly than the mixed milk of four quarters, the solids-not-fat figure of which is often an average for infected plus uninfected milk.

Experimental

Determinations were made of the total solids and fat contents, and of the casein number, of samples of milk from 247 quarters of sixty-two cows of the Shorthorn, Friesian, Ayrshire and Guernsey breeds. The samples were also examined bacteriologically for the presence of mastitis streptococci. A full description of the cows, the method of sampling, the chemical and bacteriological methods used, and the incidence of infection is given in the previous paper(2).

Discussion of results

The results are summarized in Table I. Each quarter is included once only. The solids-not-fat content has been calculated as a percentage of the fat-free milk to avoid differences which might be due solely to the presence of varying amounts of fat.

The solids-not-fat content of the uninfected samples varied from 8·24 to 10·28 and of the infected samples from 4·26 to 9·92%. The solids-not-fat of the

uninfected samples of the Shorthorn, Friesian, Ayrshire and Guernsey breeds averaged 9·36, 9·11, 9·82 and 9·83% respectively, but in the infected samples the percentages were reduced to 8·44, 8·38, 8·28 and 9·49 (one sample) showing clearly the effect of infection.

Table I. *Percentages of solids-not-fat in infected and normal samples*

Breed of cow	Number of quarter samples	Solids-not-fat % (of fat-free milk)			
		Lowest	Highest	Mean	S.D.
		Bacteriologically negative			
Shorthorn	50	8·74	10·28	9·36	0·40
Friesian	43	8·24	9·90	9·11	0·43
Ayrshire	7	9·04	10·14	9·82	0·29
Guernsey	14	9·47	10·20	9·83	0·23
		Bacteriologically positive			
Shorthorn	44	5·05	9·92	8·44	1·10
Friesian	57	4·26	9·37	8·38	0·86
Ayrshire	19	6·58	9·68	8·28	0·98
Guernsey	1	9·49			—

The figure 8·80 has been selected for the percentage of solids-not-fat in the fat-free milk which corresponds approximately with the legal presumptive minimum of 8·5% in whole milk, and the percentages of the infected and uninfected samples above and below this figure have been calculated. The results for each breed are given in Table II.

Table II. *Distribution of samples of low solids-not-fat content*

Breed of cow	Total number of quarter samples	Number of samples low in solids-not-fat	% of samples low in solids-not-fat	Average solids-not-fat for samples low in solids-not-fat	S.D.
		Bacteriologically negative			
Shorthorn	50	1	2	8·74	—
Friesian	43	9	21	8·53	0·16
Ayrshire	7	0	0	—	—
Guernsey	14	0	0	—	—
All breeds	114	10	9	8·56	0·16
		Bacteriologically positive			
Shorthorn	44	22	50	7·65	1·03
Friesian	57	40	70	8·11	0·89
Ayrshire	19	14	74	7·83	0·77
Guernsey	1	0	0	—	—
All breeds	121	76	63	7·93	0·89
		All samples (including bacteriologically doubtful)			
Shorthorn	99	24	24	7·74	
Friesian	104	49	47	8·19	
Ayrshire	28	16	57	7·91	
Guernsey	16	0	0	—	
All breeds	247	89	36	8·02	

Eighty-nine quarter samples out of the total of 247 examined (or 36%) were low in solids-not-fat. Of 121 infected samples, 76 (or 63%) were low in solids-not-fat, whilst of 114 uninfected samples only 10 (or 9%) were below the standard. Hence there are 86 samples for which a definite bacteriological result is available and of these 76 (or 88%) were infected.

The ten samples that were low in solids-not-fat content and uninfected averaged 8·56% solids-not-fat (range 8·24–8·74) showing that the deficiency was slight. Nine of these samples were from Friesian cows and two cows produced six of them. In contrast to these physiologically low solids-not-fat samples, the 76 low pathological samples had an average solids-not-fat content of 7·93%. It may therefore be concluded that milk samples with physiologically low solids-not-fat are relatively rare and that the lack of solids-not-fat is not pronounced. Subclinical mastitis, on the other hand, definitely accounts for a very high proportion of the samples of milk low in solids-not-fat content, and produces a large decrease in that content.

The relationship between the solids-not-fat percentage, the result of the bacteriological examination, and the casein number has been shown graphically in the previous paper (p. 178). The casein number provides a measure of the intensity of infection, and it will be seen that the solids-not-fat percentage is roughly proportional to the casein number. This relationship is apparent also on comparing the four individual quarters of the same cow. For example, cow No. 31 had two infected quarters, with casein numbers 42·3 and 75·9, the solids-not-fat percentages in the milk samples being 4·26 and 8·30. The normal quarters had numbers of 80·3 and 80·4, and the percentages were 8·84 and 9·07.

Summary

The solids-not-fat content (expressed as a percentage of fat-free milk) has been determined for 247 samples of milk from the individual quarters of sixty-two cows. The samples were also examined bacteriologically for the presence of mastitis streptococci and 121 were found to be infected, 114 uninfected, and twelve "doubtful".

The solids-not-fat content of the uninfected samples varied from 8·24 to 10·28 and of the infected samples from 4·26 to 9·92. For the uninfected samples of the Shorthorn, Friesian, Ayrshire and Guernsey breeds it averaged 9·36, 9·11, 9·82 and 9·83 respectively, but in the infected samples only 8·44, 8·38, 8·28 and 9·49%.

Of the 247 samples, 36% were below 8·80 in solids-not-fat content. Of 121 infected samples, 63% were below while of the 114 uninfected samples only 9% were below this standard. Hence 88% of the samples below 8·80 were infected samples, thus showing that subclinical mastitis accounts for a very high percentage of samples low in solids-not-fat content. In these samples the average value for solids-not-fat was 7·93%. The few uninfected samples that were low in solids-not-fat averaged 8·56%.

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188. THE EFFECT OF COMMERCIAL STERILIZATION ON THE NUTRITIVE VALUE OF MILK[1]

V. THE EFFECT OF COMMERCIAL STERILIZATION ON THE VITAMIN C OF MILK

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Autoclaved milk is so deficient in vitamin C that it is usually included in scurvy-producing diets for guinea-pigs. In the commercial sterilization of milk temperatures above the boiling-point of water are reached, and one would hardly expect much of the factor to survive such drastic heat treatment.

Experimental

The chemical method of estimation of vitamin C was used by us in this study, and the reduced and reversibly oxidized ascorbic acids present in milk before and after heating were measured by methods which have already been fully described elsewhere (1,2).

Table I gives the results of the analyses of fifteen samples of raw and of fifteen corresponding samples of sterilized milk. The figures are remarkable in that they show that sterilization has destroyed only about half (43%) of the total (reduced and reversibly oxidized) ascorbic acid originally present in the milk. This unexpected result led us to repeat a number of estimations by another method, that of Emmerie & van Eekelen (3), but very much the same values were obtained. Moreover, the sterilized milk behaved quite normally on exposure to light, i.e. the exposed milk gave no titration with the indophenol reagent. It is therefore most likely that our figures represent actual ascorbic acid and that the titrations have not been complicated by any non-specific reduction of the reagent by substances formed in the course of heating of the milk.

Such substances are known to be formed when milk is autoclaved at 120° C. for 1 or 2 hr. in open bottles. The autoclaved milk gives with the indophenol reagent a higher titration than the unheated milk, but the spurious substance is not destroyed on exposure to light.

As described in the first paper of this series on p. 4 the sterilization of milk after the preliminary heating is carried out anaerobically. This probably explains why a relatively large quantity of vitamin C survives the severe heat treatment.

Table I also shows that the sterilized milk contains, relatively to the raw milk, much less of the reduced ascorbic acid than of the reversibly oxidized

[1] Earlier papers (I to IV) appeared in the previous number of this volume of the *Journal of Dairy Research*.

form, and the last column of the table gives a figure of over 70 for the percentage destruction of the reduced ascorbic acid as a result of sterilization. This result seems, at first, difficult to reconcile with the known greater stability of the reduced form (1,2). It is doubtless simply due to the exposure to light after the heat treatment of the bottles containing the sterilized milk. When it reached our laboratory the sterilized milk was, in fact, at a disadvantage in comparison with the raw milk, which was kept throughout in Thermos flasks

Table I. *Vitamin C content of raw and of commercially sterilized milks*

	Raw milk mg. vitamin C per 100 ml.		Sterilized milk mg. vitamin C per 100 ml.		Percentage destruction	
Date	Total (reversibly oxidized and reduced)	Reduced	Total (reversibly oxidized and reduced)	Reduced	Total	Reduced
30. xi. 36	1·368	0·795	1·035	0·536	24·34	32·58
1. xii. 36	2·217	1·552	1·330	0·444	40·01	71·39
2. xii. 36	1·570	0·887	1·035	0·296	34·08	66·63
3. xii. 36	1·774	1·090	0·887	0·307	50·00	71·83
4. xii. 36	1·552	0·813	0·776	0·222	50·00	72·69
7. xii. 36	1·986	1·526	0·772	0·147	61·13	90·37
8. xii. 36	2·022	1·471	1·066	0·441	49·06	70·02
9. xii. 36	1·948	1·416	0·956	0·239	50·92	83·12
10. xii. 36	1·728	1·104	0·919	0·294	34·03	73·37
11. xii. 36	1·728	1·085	1·140	0·368	34·03	66·08
15. xii. 36	1·731	1·091	0·804	0·247	53·55	77·36
16. xii. 36	1·978	1·195	1·113	0·247	43·73	79·33
17. xii. 36	1·855	0·969	1·072	0·330	42·21	65·94
18. xii. 36	2·019	0·989	1·319	0·330	34·67	66·63
21. xii. 36	2·022	1·245	1·245	0·331	38·43	73·41
Average	1·833	1·149	1·031	0·315	42·68	70·72
S.E.M.	±0·058	±0·064	±0·047	±0·026	±2·52	±3·13

in complete darkness. A single sample of sterilized milk which had been wrapped in black paper immediately after sterilization and brought to the laboratory without exposure to light gave a total titration of 1·34 mg.% and had a reduced ascorbic acid content of 1·08 mg.%, i.e. double that of the highest sample of sterilized milk given in Table I.

Both the total and the reduced ascorbic acid values of the raw milk used in the present study are definitely lower than corresponding figures for the raw milk used in our work on pasteurized milk (1), and even more so than values found by us for the "sale" milk from this Institute (4). This decrease in the vitamin C content of raw milk can be correlated with and is most probably due to the extent to which the milk is handled in the process of bulking and to the time taken in transport.

It has already been mentioned in the third paper of this series (5) that several samples of sterilized milk were stored by us in a dark cupboard for from 4 to 6 weeks. These were analysed for vitamin C after storage. The results are given in Table II. Unfortunately, only in one sample, that of milk of 15 December, were tests carried out before and after storage, otherwise the samples were different. However, a comparison of the average figures shows

that storage for the time specified has resulted in a further decrease by some 30% of the vitamin C content of the sterilized milk.

Table II. *Vitamin C content of milks which had been stored for a month or 6 weeks*

		mg. vitamin C per 100 ml.	
Date received	Date analysed	Total (reversibly oxidized and reduced)	Reduced
4. xi. 36	7. xii. 36	1·066	0·221
5. xi. 36	7. xii. 36	0·919	0·147
16. xi. 36	5. i. 37	0·417	0·256
17. xi. 36	5. i. 37	0·497	0·288
23. xi. 36	5. i. 37	0·641	0·273
24. xi. 36	5. i. 37	0·561	0·256
14. xii. 36	18. i. 37	0·917	0·220
15. xii. 36	18. i. 37	0·605	0·220
	Average	0·703	0·235

SUMMARY

1. Fifteen samples of raw and fifteen samples of commercially sterilized milk from the same bulk were analysed for vitamin C by the chemical method (titration with dichlorophenol-indophenol).

2. The raw milk contained on an average 1·83 mg./100 ml. of total (reduced and reversibly oxidized) ascorbic acid. The corresponding figure for sterilized milk was 1·03 mg./100 ml., a loss of 43% of the original value.

3. Storage of the sterilized milk for a period of 4–6 weeks resulted in a further reduction equivalent to about 30%.

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189. THE EFFECT OF COMMERCIAL STERILIZATION ON THE NUTRITIVE VALUE OF MILK

VI. COMPARISON OF THE TOTAL NUTRITIVE VALUE OF RAW AND COMMERCIALLY STERILIZED MILKS

By K. M. HENRY, E. W. IKIN and S. K. KON

(With 4 Figures)

In previous communications(1,2) we expressed the opinion that when milks supplemented with minerals are fed in equalized amounts and as the sole diet to groups of rats, the high level of intake tends to mask differences due to season or caused by pasteurization. It was of interest to find out whether under similar experimental conditions the more drastic effects of commercial sterilization would be detectable.

In the earlier work(2) we had obtained promising results when the intake of milk was cut down and a basal deficient diet was offered to the rats as an additional source of protein, salts and calories. In such experiments the rats grew subnormally, but a marked improvement followed the addition of yeast, suggesting a deficiency in the original diet of one or more of the components of the vitamin B complex. Here again raw milk gave the same results as pasteurized milk, but it seemed likely that the method could be used with advantage to gauge the effects of a more severe heat treatment.

For this reason we planned to feed to groups of rats raw or sterilized milk in addition to either a basal diet deficient (among other factors) in the vitamin B complex, or else to the same diet supplemented with yeast. Lastly, we decided to add only sugar and minerals to the raw and sterilized milks. In this way the caloric value of the milks was increased. Consequently the intakes of the milks and therefore of their constituent factors were cut down.

We hoped again that this method would be more likely to show any limitations of sterilized milk than a method involving the feeding of larger quantities of milks to which only minerals are added.

The following experiments were therefore carried out:

(1) In this experiment the raw and sterilized milks supplemented with iron, copper and manganese were fed as an exclusive diet.

(2) Limited quantities of the two milks were fed in addition to a basal diet supplying only energy, protein and salts.

(3) Similar quantities of the milks were fed but yeast was added to the basal diet. The yeast probably supplied other factors in addition to the various components of the vitamin B complex.

(4) Cane sugar was dissolved in the milks to increase their caloric value. The milks were then fed as in the first experiment. Two separate experiments were done, the quantity of sugar used in one being double that used in the other.

The results of these experiments are described in detail in the following pages.

Experimental

(i) *Exclusive diet of milk supplemented with minerals*

Twelve pairs of litter-mate male rats were placed, when 21–24 days old, in individual cages. The rats were from seven litters and weighed from 47 to 66 g. They were allotted by toss of coin to either raw or sterilized milk. Details of the technique of feeding will be found elsewhere (1,2). The milk intake was equalized within pairs. The experiment lasted from 21 September until 18 December 1936; the rats were then killed by coal gas and measured in the usual way.

Results.

Fig. 1 shows the average growth curves for the two groups of experimental animals. It will be seen that the two curves lie very close together throughout the experiment. Table I gives individual and average figures for the weights of the animals, for the gains in weight, total and daily, for milk intakes and milk refusals at 8 weeks and at 12 weeks; it also gives the body lengths of the rats at the end of the experiment. Table II gives the statistical treatment of the differences in gains in weight, in the milk refusals at 8 and 12 weeks, and in the body lengths of the two groups of rats.

Table I shows that at 8 weeks the gain in weight of the rats on raw milk was slightly superior to that of the sterilized milk group, the actual figures being 132·1 and 129·8 g. respectively, though Table II shows that this difference is statistically not significant. As the difference was so small it was decided to continue the experiment for a further 4 weeks. At the end of this period the gains in weight were 161·4 and 163·3 g. for raw and sterilized milk respectively. This difference, now slightly in favour of the sterilized milk, is also without statistical significance. The average body lengths of the rats in the two groups were almost identical, and the difference is obviously not statistically significant.

The average daily gain in weight of 2·3 g. in the course of 8 weeks is satisfactory for an experiment of this type and is very similar to the gain shown by rats in a previous experiment with pasteurized milk (1). At 12 weeks the average daily gain had fallen to 1·9 g. This is accounted for by the normal slowing up of the rate of growth. Such cessation of rapid growth usually occurs in male rats on the stock ration at about this age.

The average milk intakes of the two groups are very similar both at 8 and 12 weeks. The small differences are attributable to the fact that some rats

(notably those on raw milk) left milk on the last day of the period. Throughout the experiment it was noticed that in most cases the sterilized milk group consumed their milk more eagerly than their litter-mates receiving the raw milk. It will be seen from Table II that the difference is statistically highly significant, as it is shown even by a method which tends to underestimate the value of such differences (3).

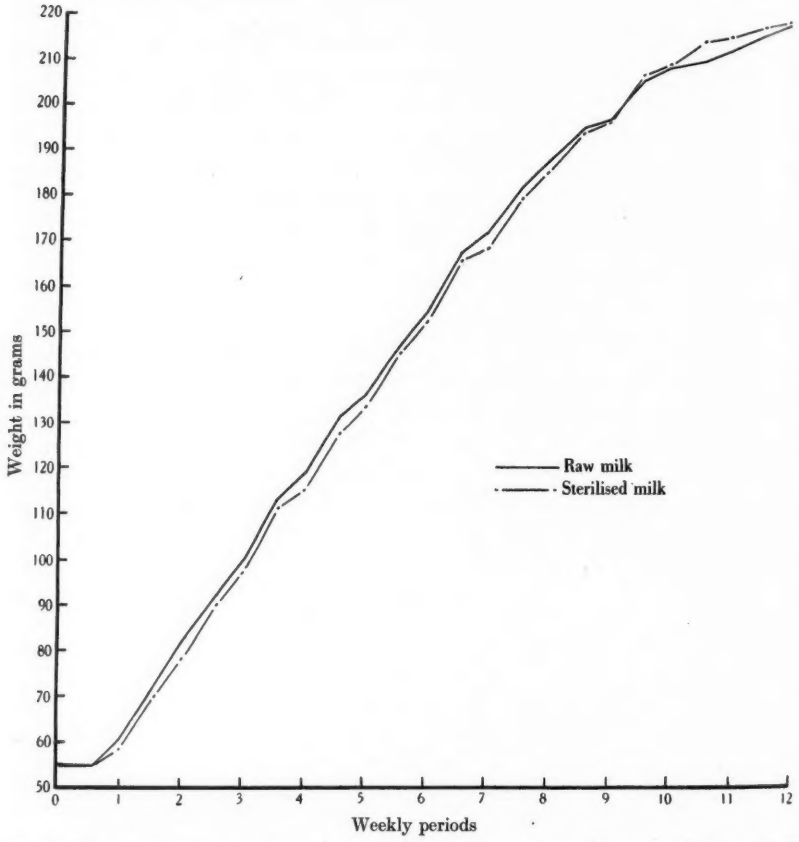

Fig. 1. The growth of rats on an exclusive milk diet. Average growth curves (12 ♂♂ each) of rats receiving an exclusive diet of either raw or sterilized milk supplemented with minerals. Milk intakes equalized within groups. Duration of experiment 12 weeks.

(ii) *Milk fed with a diet supplying energy, protein and salts, but deficient in other respects*

The diet and technique used in this experiment were practically the same as those described in a previous publication (2), diet 68 A being used throughout the feeding period.

Table I. *Weights; gains in weight; daily gains in weight; body lengths and milk intakes and refusals of the experimental rats on exclusive milk diets*

Litter no.	Rat no.	Weight (g.)			Gain (g.)		Gain/day (g.)		Milk intake (ml.)		Body length when killed (mm.)	No. of milk refusals	
		Initial	8 weeks	12 weeks	8 weeks	12 weeks	8 weeks	12 weeks	8 weeks	12 weeks		8 weeks	12 weeks
Raw milk													
I	5743	47	139	200	92	153	1·64	1·82	3090	5436	209	44	61
II	5769	57	213	252	156	195	2·79	2·32	4475	7145	222	5	14
III	5821	54	193	222	139	168	2·48	2·00	4110	6665	213	33	55
IV*a*	5834	51	171	194	120	143	2·14	1·70	3964	6485	211	32	53
IV*b*	5832	54	193	218	139	164	2·48	1·96	4176	6720	220	29	46
IV*c*	5831	57	189	206	132	149	2·36	1·77	4240	6585	216	19	20
V*a*	5848	52	203	230	151	178	2·70	2·12	4361	7044	221	28	43
V*b*	5847	59	219	254	160	195	2·86	2·32	4785	7730	229	23	40
VI*a*	5870	56	180	186	124	130	2·21	1·55	3777	6010	212	35	58
VI*b*	5868	54	212	235	158	181	2·82	2·15	4145	6715	218	19	37
VI*c*	5871	55	150	190	95	135	1·70	1·61	3584·5	5859	203	29	46
VII	5876	66	185	212	119	146	2·13	1·74	4290	7070	217	4	7
Average		55·2	187·3	216·6	132·1	161·4	2·36	1·92	4083·1	6622·0	215·9	25·0	40·0
Sterilized milk													
I	5740	48	147	193	99	145	1·77	1·73	3130	5435	206	0	0
II	5767	56	205	232	149	176	2·66	2·10	4471	7145	218	22	31
III	5822	58	203	234	145	176	2·59	2·10	4110	6665	215	0	0
IV*a*	5828	53	169	205	116	152	2·07	1·81	3965	6485	213	1	1
IV*b*	5829	55	184	215	129	160	2·30	1·90	4180	6720	215	6	8
IV*c*	5833	56	179	195	123	139	2·20	1·65	4240	6578	209	17	37
V*a*	5844	52	200	232	148	180	2·64	2·14	4370	7045	221	0	1
V*b*	5846	56	215	251	159	196	2·84	2·33	4785	7730	227	6	8
VI*a*	5867	56	187	216	131	160	2·34	1·90	3780	6040	212	0	0
VI*b*	5869	54	199	232	145	178	2·59	2·12	4145	6715	218	14	15
VI*c*	5872	56	145	192	89	136	1·59	1·62	3590	5865	211	2	2
VII	5877	60	185	222	125	162	2·23	1·93	4282·5	7067	213	30	44
Average		55·0	184·8	218·3	129·8	163·3	2·32	1·94	4087·4	6624·2	214·8	8·2	12·2

Twelve pairs of newly weaned litter-mate male rats from six litters weighing 50–59 g. were placed in individual cages. They were allocated by toss of coin to either raw or sterilized milk. The rats were given diet 68A *ad libitum* throughout the experiment which lasted from 18 September to 13 November 1936. During the first 3 days the rats were given only the diet and water. On the fourth day all animals received 5 ml. of the appropriate milk. As this was readily consumed, they all were given 10 ml. on the second day, while for the next 3 days all received 15 ml. daily. After that, with the exception of Sundays, all rats were given 20 ml. of milk daily.

Table II. *Statistical treatment of average differences in gains in weight (at 8 and 12 weeks); in body lengths and in milk refusals (at 8 and 12 weeks) of the experimental rats on exclusive milk diets*

	Gain in weight over 8 weeks (g.)	Gain in weight over 12 weeks (g.)	Body length when killed (mm.)
Average: raw milk	132·1	161·4	215·9
Average: sterilized milk	129·8	163·3	214·8
Difference: Raw – sterilized	+2·3	−1·9	+1·1
Standard error of the mean (S.E.M.)	±2·08	±3·71	±1·15
*P**	1 : 3	1 : 2	1 : 3
	Not significant	Not significant	Not significant

	No. animals refusing more frequently on specified milk	
	8 weeks	12 weeks
Raw milk	10	9
Sterilized milk	2	3
Difference from mean	4	3
Standard error (S.E.)	±1·19	±1·5
*P**	1 : 1000	1 : 25
	Significant	Significant

* *P* = probability that a difference at least as great as the observed difference would have arisen by random sampling from a homogeneous population.

Results.

Composite growth curves for the two groups of rats are shown in Fig. 2. It will be seen that little or no growth occurred in either group until milk was given. After only a week on the milk the growth of the rats on raw milk was very definitely superior to the growth of those on sterilized milk. Table III gives individual figures for weights, gains in weight, daily gains, intakes of diet, milk and total solids, gains per g. solids ingested, and body lengths of the rats in both groups. The total and daily gains shown by the raw-milk group were nearly double those shown by the sterilized milk group. The average daily gain of 2·02 g. of the rats receiving raw milk is higher than in similar experiments reported in our previous paper (2), but is still rather low. This is probably attributable to the fact that, although the diet was fed *ad libitum*, its marked deficiency in various factors was not quite made good by the limited milk intake. The daily gain of the sterilized-milk rats was very markedly inferior, being only 1·2 g. These rats showed diminished appetite and ate much less of

the supplementary diet. It will also be noticed that the gains in weight of the sterilized-milk rats were less economical as they gained only 0·16 g. per g. of solids eaten against a gain of 0·21 g. by the raw-milk rats. Most probably at their low rate of growth and diminished food intake they used a larger part of the diet to cover their maintenance requirements. The differences between the two groups of rats are so marked that statistical tests of significance are unnecessary.

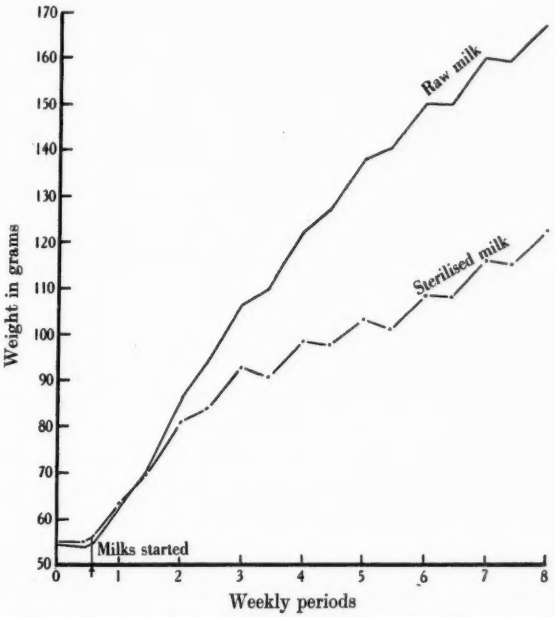

Fig. 2. The growth of rats on a deficient diet supplemented with milk. Average growth curves (12 ♂♂ each) of rats receiving 20 ml. daily of either raw or sterilized milk in addition to a basal diet supplying only protein, energy and salts.

The rats were weighed twice a week, on Mondays and Fridays. After the second week of the experiment the weights of the rats receiving sterilized milk showed no increase and frequently a slight loss at the Monday weighing. This is probably linked up with the fact that the rats were given no milk on Sundays, and suggests a borderline deficiency in the vitamin B complex of the sterilized milk. Similar fluctuations on diets low in the vitamin B complex have been noticed by Kon *et al.* (5).

(iii) *Milk fed with a diet supplying the vitamin B complex in addition to protein energy and salts*

Ten pairs of litter-mate male rats from six litters were placed, when 21–22 days old and weighing 48–57 g., in individual cages. They were, as usual, allocated to raw or sterilized milk by toss of coin. They were given diet 69

Table III. *Weights; gains in weight; daily gains in weight; intakes of basal diet, milk and total solids; gains/g. solids ingested and body lengths of experimental rats receiving raw or sterilized milk in addition to a basal deficient diet (diet 68A) supplying only protein, energy and minerals*

Litter no.	Rat no.	Weight (g.) Initial	Weight (g.) Final	Gain (g.)	Gain/day (g.)	Basal diet intake (g.)	Milk intake (ml.)	Total solid* intake (g.)	Gain/g. solids ingested (g.)	Body length (mm.)
					Raw milk					
I*a*	5722	53	184	131	2·34	437·92	860	542·51	0·2415	205
I*b*	5724	56	177	121	2·16	450·23	860	554·59	0·2182	200
II*a*	5733	55	169	114	2·04	442·03	860	546·55	0·2086	194
II*b*	5734	56	159	103	1·84	411·43	860	516·54	0·1994	193
III*a*	5752	55	180	125	2·23	432·90	860	537·59	0·2325	200
III*b*	5755	56	171	115	2·05	500·94	860	604·31	0·1903	200
IV	5766	50	177	127	2·27	472·89	860	576·81	0·2202	198
V*a*	5780	50	158	108	1·93	443·95	860	548·43	0·1969	191
V*b*	5782	50	168	118	2·11	413·04	860	518·12	0·2278	195
VI*a*	5786	59	156	97	1·73	412·32	860	517·41	0·1875	192
VI*b*	5787	55	163	108	1·93	426·36	860	531·18	0·2033	192
VI*c*	5790	56	146	90	1·61	387·54	860	493·11	0·1825	184
Average		54·2	167·3	113·1	2·02	435·96	860	540·59	0·2091	195·3
					Sterilized milk					
I*a*	5721	56	115	59	1·05	265·73	860	373·67	0·1579	180
I*b*	5723	57	135	78	1·39	291·38	860	398·82	0·1956	190
II*a*	5735	55	105	50	0·89	286·70	860	394·23	0·1268	169
II*b*	5736	56	100	44	0·79	278·08	860	385·76	0·1141	179
III*a*	5751	56	121	65	1·16	289·35	860	396·83	0·1638	181
III*b*	5754	56	129	73	1·30	295·73	860	403·08	0·1811	185
IV	5768	54	129	75	1·34	297·23	860	404·55	0·1854	180
V*a*	5781	50	134	84	1·50	343·46	860	449·89	0·1867	180
V*b*	5783	51	147	96	1·71	331·59	860	438·25	0·2191	189
VI*a*	5791	58	113	55	0·98	270·21	860	378·06	0·1455	176
VI*b*	5788	56	131	75	1·34	352·32	860	458·58	0·1636	182
VI*c*	5789	56	114	58	1·04	313·86	860	420·86	0·1378	174
Average		55·1	122·8	67·7	1·21	301·30	860	408·55	0·1648	180·4

* Calculated on dry matter. Solids in milk are calculated from the data in Table I of the first paper of this series (4), on the assumption that the specific gravity of milk is 1·03. Moisture in diet 68A = 1·94%.

(i.e. 95 parts of diet 68A + 5 parts of brewer's yeast) *ad libitum* throughout the experiment. After 4 days on the basal diet alone each rat was given 5 ml. of either raw or sterilized milk, on the next 2 days the milk ration was successively raised to 10 ml. and 15 ml. As some of the rats were disinclined to clear their milk each day, the daily intake was not raised to 20 ml. until the beginning of the third week on milk. If the daily ration of milk was not completely taken by the following morning, the amount remaining was measured back and a corresponding amount was offered in addition to that day's ration. The experiment lasted from 19 September to 14 November 1936.

Results.

The composite growth curves for the two groups of rats are shown in Fig. 3. It will be seen that good growth started as soon as the animals were placed on the diet. The rate of growth was steady throughout the experiment. As opposed to the findings reported in the previous section (diet 68A) the addition of milk to the diet had no noticeable effect on the growth rate. Table IV gives individual figures for the weights, gains in weight, daily gains in weight, intakes of basal diet, milk and total solids, gains per g. solids ingested and body lengths of the two groups of rats. The results of statistical tests are shown in Table V. The differences in gain in weight are very small. The slightly superior growth of the rats receiving the sterilized milk is without statistical significance. The average intake of basal diet by the sterilized group is slightly but not significantly greater than that of the raw milk group. The gain per g. solids intake is also slightly greater for the sterilized group, but once again the difference lacks statistical significance. The differences in body length are likewise statistically not significant. The average daily growth of over 3 g. for both groups was quite satisfactory.

These results show that the differences between raw and sterilized milk which were apparent in the preceding experiment were made good by the addition of yeast to the supplementary diet.

(iv) *Exclusive diet of milk supplemented with minerals and with energy in the form of sugar dissolved in the milk*

These experiments lasted for only 4 weeks, as rats were only available for this period before the supply of milk stopped.

The sugar was added to the milk at two levels, i.e. at the rate of 15 or 30 g. of sugar per 100 ml. of milk. The sugar readily dissolved on gentle shaking. 15 g. of sugar and 100 ml. of milk yielded 110 ml. of solution. The higher level of sugar gave 120 ml.

Twelve pairs of newly-weaned male rats from nine litters weighing 39–48 g. were divided into two groups of six pairs each and placed in individual cages. The first group received milk plus the lower level of sugar. The second group were given milk containing the higher level of sugar. The rats were as usual allotted by toss of coin to either raw or sterilized milk.

On the first day each rat was given 0·3 ml. of salt mixture No. 3 of Elvehjem *et al.* (6) with 2 ml. of the appropriate milk. When this was consumed the balance of the day's ration was given. The amount of milk first offered with the salt mixture was raised to 5 ml. in the course of the first week. During the second week 0·6 ml. of the salt mixture was given daily, while in the third and fourth weeks the daily allowance was increased to 1 ml.

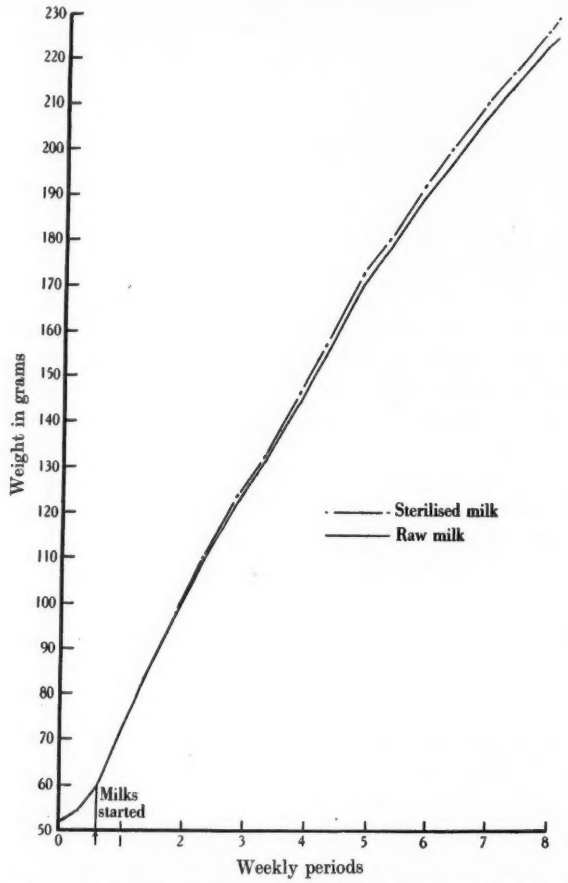

Fig. 3. The growth of rats on a diet containing yeast and supplemented with milk. Average growth curves (10 ♂♂ each) of rats receiving 20 ml. daily of either raw or sterilized milk in addition to a basal diet supplying the vitamin B complex as well as protein, energy and salts.

On the lower level of sugar the milk intake was equalized within pairs throughout the experiment. With the rats receiving the higher level of sugar, the milk intake was equalized within pairs for the first 8 days, subsequently it was fed *ad libitum* as the group on sterilized milk consistently left milk and the growth of the raw-milk group was consequently being retarded.

Table IV. *Weights; gains in weight; daily gains in weight; intakes of basal diet, milk and total solids; gains/g. solids ingested and body lengths of experimental rats receiving raw or sterilized milk in addition to a diet containing 5% brewer's yeast as well as protein, energy and minerals (diet 69)*

Litter no.	Rat no.	Weight (g.) Initial	Weight (g.) Final	Gain (g.)	Gain/day (g.)	Basal diet intake (g.)	Milk intake (ml.)	Total solid* intake (g.)	Gain/g. solids ingested (g.)	Body length (mm.)
					Raw milk					
I	5741	52	241	189	3·38	715·29	830	806·41	0·2344	212
II*a*	5760	51	253	202	3·61	703·11	830	794·54	0·2542	216
II*b*	5759	50	195	145	2·59	642·70	823†	734·73	0·1974	203
III	5810	51	223	172	3·07	650·08	830	742·84	0·2315	213
IV*a*	5817	50	205	155	2·77	579·45	830	673·99	0·2300	205
IV*b*	5818	52	166	114	2·04	478·73	830	575·81	0·1980	191
V*a*	5840	55	235	180	3·21	648·70	830	741·50	0·2428	213
V*b*	5841	50	217	167	2·98	628·43	830	721·74	0·2314	207
VI*a*	5851	56	266	210	3·75	747·91	830	838·21	0·2505	218
VI*b*	5854	57	249	192	3·43	720·28	830	811·27	0·2367	221
Average		52·4	225·0	172·6	3·08	651·47	829·3	744·10	0·2307	209·9
					Sterilized milk					
I	5744	50	233	183	3·27	672·04	830	764·25	0·2395	208
II*a*	5757	50	204	154	2·75	567·83	830	662·67	0·2324	206
II*b*	5761	48	208	160	2·86	594·98	830	689·13	0·2322	208
III	5811	48	222	174	3·11	680·44	830	772·44	0·2253	213
IV*a*	5816	51	219	168	3·00	600·28	830	694·30	0·2420	210
IV*b*	5819	51	197	146	2·61	602·02	830	695·99	0·2098	205
V*a*	5838	54	249	195	3·48	760·45	830	850·43	0·2293	213
V*b*	5839	52	225	173	3·09	647·53	830	740·36	0·2337	210
VI*a*	5852	56	263	207	3·70	718·15	830	809·20	0·2558	219
VI*b*	5853	57	275	218	3·89	804·27	830	893·15	0·2441	220
Average		51·7	229·5	177·8	3·18	664·80	830	757·19	0·2344	211·2

* Calculated on dry matter. Solids in milk are calculated from the data in Table I of the first paper of this series (4), on the assumption that the specific gravity of milk is 1·03. Moisture in diet 69 = 2·52 %.

† 7 ml. of milk left on the last day of the experiment.

Results.

The growth curves for the groups of experimental animals are shown in Fig. 4. Individual figures for the weights, gains in weight, intakes of the milk plus sugar solution, milk, sugar and total solids, body lengths and milk refusals of the experimental rats will be found in Table VI, while Table VII gives the statistical treatment of some of the differences between the two groups of rats on the lower level of sugar.

Table V. *Statistical treatment of average differences in gains in weight; in gains/g. solids ingested; in basal diet intake and in body lengths of rats receiving milk in addition to diet* 69

	Gain in weight (g.)	Gain/g. solids ingested (g.)	Basal diet intake (g.)	Body length (mm.)
Average raw milk	172·6	0·2307	651·47	209·9
Average sterilized milk	177·8	0·2344	664·80	211·2
Difference: raw – sterilized	−5·2	−0·0037	−13·33	−1·3
S.E.M.	±7·02	±0·0049	±25·39	±1·99
*P**	1 : 2	1 : 2	1 : 2	1 : 2
	Not significant	Not significant	Not significant	Not significant

* *P* = probability that a difference as great as the observed difference would have arisen by random sampling from a homogeneous population.

(*a*) *The lower level of sugar.* Fig. 4 shows that after the first few days on the diet both groups of rats fed with the lower level of sugar in their milk maintained good growth, amounting to nearly 3 g. per day over a period of 4 weeks (Table VI). During the last 4 days of the experiment, however, the raw-milk rats went decidedly ahead.

Table VII shows that the difference between the actual gains on the two milks was statistically significant. As the total solids in the experimental milks (Table I of the first paper of this series(4)) showed some variations during the experimental period, the gains per g. solids ingested were calculated. Here again the differences were statistically significant. The odds were 44 : 1 and 58 : 1 respectively against the differences being due to chance. The differences between the body lengths of the two groups were not significant, the odds being 8 : 1 in favour of the raw-milk group. Throughout the experiment all six rats on sterilized milk refused their milk more frequently than their littermates (Table VI), which shows that their appetite had been definitely affected. Statistical tests were obviously unnecessary.

(*b*) *The higher level of sugar.* Fig. 4 shows that there was an initial drop in weight for both the raw and the sterilized groups receiving the higher level of sugar in their milk. At the end of 8 days the raw-milk rats had regained their initial weight; they were subsequently given milk *ad libitum* (see p. 196), and thereafter their rate of growth was similar to that of the animals which received the lower level of sugar. The growth of the rats given sterilized milk was very markedly inferior to that of the raw-milk group. One of these rats (No. 6010)

Table VI. *Weights; gains in weight; daily gains in weight; intakes of milk+sugar solution, milk, sugar and total solids; gains/g. solids ingested; body lengths and milk refusals of rats receiving raw or sterilized milk plus* (*a*) 15 *g.*

Table VI. *Weights; gains in weight; daily gains in weight; intakes of milk + sugar solution, milk, sugar and total solids; gains/g. solids ingested; body lengths and milk refusals of rats receiving raw or sterilized milk plus (a) 15 g. sugar/100 ml. milk, (b) 30 g. sugar/100 ml. milk*

Litter no.	Rat no.	Weight (g.)		Gain (g.)	Gain/day (g.)	Food intake				Gain/g. solids ingested (g.)	Body length (mm.)	No. of refusals
		Initial	Final			Milk + sugar solution (ml.)	Milk (ml.)	Sugar (g.)	Total* solids (g.)			
Raw milk + first level of sugar (15 g./100 ml.)												
I*a*	5995	48	142	94	3·36	1230·0	1118·2	167·7	315·6	0·2978	185	5
II	5999	46	126	80	2·86	980·0	890·9	133·6	251·5	0·3181	173	1
III*a*	6003	40	126	86	3·07	1040·0	945·5	141·8	266·9	0·3222	175	11
V	6007	39	100	61	2·18	795·0	722·7	108·4	204·0	0·2990	169	2
VII	6012	41	147	106	3·79	1274·0	1158·2	173·7	326·9	0·3243	183	4
IX*a*	6015	44	108	64	2·29	1035·0	940·9	141·1	265·6	0·2410	170	4
Average		43·0	124·8	81·8	2·93	1059·0	962·7	144·4	271·8	0·3004	175·8	4·5
Sterilized milk + first level of sugar (15 g./100 ml.)												
I*a*	5996	48	144	96	3·43	1229·5	1117·8	167·7	317·2	0·3026	181	16
II	6000	45	113	68	2·43	978·0	889·1	133·4	252·4	0·2694	169	19
III*a*	6004	39	122	83	2·96	1040·0	945·5	141·8	268·3	0·3094	178	12
V	6008	39	92	53	1·89	793·0	720·9	108·1	204·6	0·2590	157	23
VII	6011	43	141	98	3·50	1280·0	1163·6	174·6	330·3	0·2697	180	18
IX*a*	6016	44	100	56	2·00	1022·0	929·1	139·4	263·7	0·2124	168	23
Average		43·0	118·7	75·7	2·70	1057·1	961·0	144·2	272·8	0·2749	172·2	18·5
Raw milk + second level of sugar (30 g./100 ml.)												
I*b*	5997†	45	115	70	2·50	831·5	692·8	207·9	299·6	0·2336	168	
III*b*	6001	41	111	70	2·50	766·0	638·3	191·5	275·9	0·2537	169	
IV	6005	44	94	50	1·79	697·5	581·3	174·4	251·3	0·1986	164	
VI	6009†	40	89	49	1·75	664·0	553·3	166·0	239·2	0·2048	158	
VIII	6014	45	116	71	2·53	803·0	669·2	200·7	289·2	0·2455	180	
IX*b*	6017	43	94	51	1·82	773·5	644·6	193·4	278·7	0·1830	161	
Average		43·3	103·2	60·5	2·16	760·0	633·4	190·0	273·8	0·2202	168·5	
Sterilized milk + second level of sugar (30 g./100 ml.)												
I*b*	5998	45	Dead	—	—	—	—	—	—	—	—	
III*b*	6002	43	80	37	1·32	535·5	446·3	133·9	193·6	0·1911	154	
IV	6006	43	78	35	1·25	571·5	476·3	142·9	206·6	0·1694	153	
VI	6010	42	Dead	—	—	—	—	—	—	—	—	
VIII	6013	46	77	31	1·11	556·5	463·8	139·1	201·2	0·1541	149	
IX*b*	6018	43	61	18	0·64	534·5	445·4	133·6	193·2	0·0932	145	
Average		43·8	74·0	30·2	1·08	549·5	458·0	137·4	198·7	0·1520	150·3	

* Solids in milk are calculated from the data in Table I of the first paper of this series (4) on the assumption that the specific gravity of milk is 1·03.

† The figures for these rats are omitted from the averages as their litter-mates died before the end of the experiment.

Table VII. *Statistical treatment of average differences in gains in weight; in gains/g. solids ingested and in body lengths of rats receiving milk plus 15 g. sugar/100 ml.*

	Gain in weight (g.)	Gain/g. solids ingested (g.)	Body length (mm.)
Average: raw milk	81·8	0·3004	175·8
Average: sterilized milk	75·7	0·2749	172·2
Difference: raw – sterilized	+6·1	+0·0255	+3·6
S.E.M.	±2·01	±0·0078	±1·98
*P**	1 : 44	1 : 58	1 : 8
	Significant	Significant	Not significant

* *P* = probability that a difference as great as the observed difference would have arisen by random sampling from a homogeneous population.

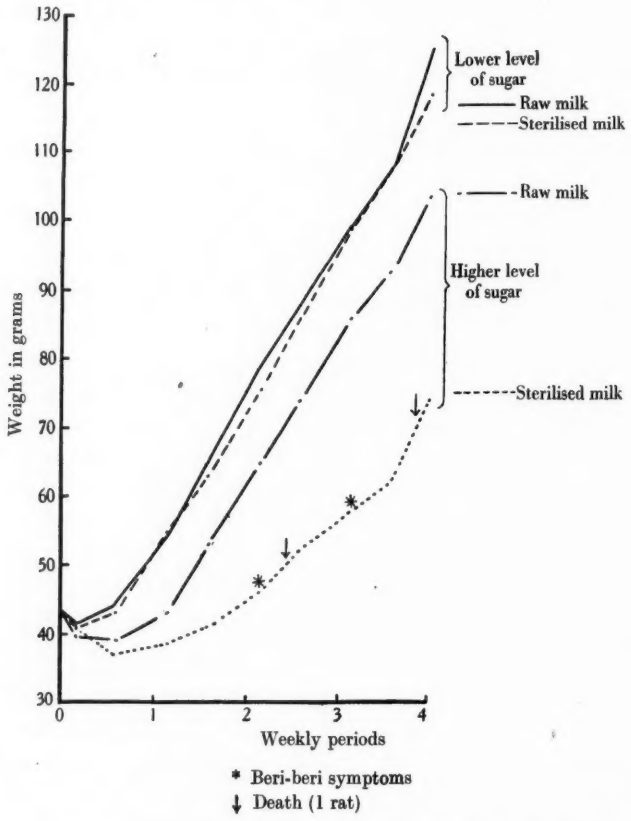

Fig. 4. The growth of rats on milk to which sugar had been added. Average growth curves (6 ♂♂ each) of rats receiving either raw or sterilized milk supplemented with 15 and 30 g. of sugar per 100 ml. of milk.

developed severe symptoms of beri-beri on the 15th day and was found dead 2 days later; on the 22nd day a second rat (No. 5998) also developed beri-beri symptoms, and was found dead in its cage on the 23rd day of the experiment. The raw-milk rats gained over 2 g. daily or twice as much as their litter-mates. These very marked differences in gains in weight render statistical treatment unnecessary. The total solid intake of the "sterilized" group was also markedly lower than that of the "raw" group (Table VI), and even when the gain in weight per g. solids intake is calculated the superiority of the raw milk rats is still evident. The rate of growth of the sterilized milk rats was probably too low for economical gains.

General discussion

The several experiments described above were not designed to investigate the fate of an isolated factor or component of milk in the course of sterilization, but were concerned with the general effect of the raw or sterilized milk on the growth of the experimental animals. In other words they were yielding information as to the total nutritive value of the milks. For this reason the results of all these experiments are best considered together.

Table VIII. *Average daily milk intakes of the rats in the various experiments on the total nutritive value of raw and sterilized milk*

Type of experiment	Average daily milk intake (ml.)
Exclusive diet of milk + minerals: 8 weeks	72·9
Exclusive diet of milk + minerals: 12 weeks	78·8
Milk + diet 68A: whole experiment, 8 weeks	15·3
Milk + diet 68A: from time milk was given, 52 days	16·5
Milk + diet 69: whole experiment, 8 weeks	14·8
Milk + diet 69: from time milk was given, 52 days	16·0
Milk (raw and sterilized) + minerals + lower level of sugar, 4 weeks	34·4
Sterilized milk + minerals + lower level of sugar, last 20 days	38·7
Raw milk + minerals + higher level of sugar, 4 weeks	22·6
Raw milk + minerals + higher level of sugar, last 20 days	26·6
Sterilized milk + minerals + higher level of sugar, 4 weeks	16·4

In doing so the levels of milk intake may serve as a useful basis for comparison. In Table VIII we have entered the average daily consumption of milk by the rats in the different experiments. As would be expected, the rats which were given nothing but minerals in addition to their milk consumed by far the largest amount. They took between 70 and 80 ml. of milk daily. It would seem that this level provided a generous margin for the factors known to be present in milk, and that this margin was wide enough to cover the losses sustained by milk in the course of commercial sterilization which have been described by us in the preceding pages.

The problem of appetite in equalized feeding experiments has been discussed at length elsewhere (2,3), and its importance for a satisfactory interpretation of experiments of this type has been emphasized. It is rather unexpected to find that in the present experiment rats receiving the sterilized milk supplemented with minerals took the milk more readily than their litter-mates on

raw milk. This finding, supported by statistical evidence, allows us to assume that, given unlimited access to milk, the sterilized milk animals would have made better gains. Such a result could, however, hardly be taken as showing an improvement in the nutritive qualities of milk as a result of the heat treatment. All it does, in our opinion, is to suggest that in exclusive milk feeding the level of intake is too generous to yield satisfactory comparisons even in experiments where one of the substances under comparison has suffered quite definite losses. One is tempted to think that at such luxury levels of feeding questions of flavour and taste may exert a definite influence.

Next in the order of quantity of milk taken come the experiments in which 15 g. of sugar were added to every 100 ml. of milk. This addition increased the caloric value of a given volume 1·7 times, and we find accordingly that the consumption has dropped very considerably when compared with that of the rats receiving only milk and minerals. The daily average is less than half that in the previous experiment in which, however, the average was taken over a period of 8 weeks. The sugar experiment lasted only 4 weeks, the rats were smaller and needed correspondingly less milk.

Halving the supply of the various essential factors brought the level of milk feeding to just that stage when the differences between raw and sterilized milk became manifest, though it was not until the end part of the experiment that actual differences in growth rate were observed.

The next lower stage in the level of milk intake was reached by the rats receiving the higher level of sugar dissolved in raw milk. The caloric value of this mixture was 2·2 times that of the original milk. As a result of the further lowering of the intake of the various essential factors the difference between raw and sterilized milk became so obvious that it was necessary to discontinue after a time the equalized feeding technique. To this the raw-milk rats responded by an immediate quickening of growth, and thereafter their growth curve ran almost parallel to the growth curves of the two groups of rats receiving the lower level of sugar. From this fact it is possible to make certain deductions concerning the nature of the nutritive difference between the raw and heated milk shown in these experiments.

It will be recalled that rats receiving the higher level of sugar dissolved in sterilized milk showed definite symptoms of vitamin B deficiency, and it therefore seems likely that the partial destruction of this factor was responsible for the differentiation of the two milks at the lower level of sugar. In the latter experiment the milk intakes were equalized, but as the appetite of the sterilized milk rats was lowered and the intake was mostly governed by their level of consumption (p. 198), they were actually receiving the milk almost *ad libitum*. For the last 3 weeks of the experiment the rats on raw milk and the higher level of sugar were also not restricted in their intake. The almost identical shape of the growth curves of these two groups suggests that in both cases growth was governed by one or more common limiting factors.

We have summarized in Table IX the figures for the last 20 days of these

two experiments.[1] From this it will be seen that the groups made almost the same gains in weight and consumed almost exactly the same quantities of total solids and calories. The milk intakes, however, differed, and the sterilized milk rats took 1·4 times the amount consumed by the raw-milk group. As the sugar presumably supplied only energy one must look to the milks for the supply of essential factors governing the appetite and the utilization of food, and it appears that 1 ml. of raw milk was in this respect equivalent to 1·4 ml. of the sterilized product. Now it will be remembered that in experiments on the vitamin B_1 content of sterilized milk we found (8) that it had lost some 30% of the amount originally present in the raw milk. As a source of vitamin B_1, the 38·7 ml. of sterilized milk taken daily by the group of animals on sterilized milk would be equivalent to only 27·1 ml. of raw milk. The closeness of the agreement between this figure and the actual intake of the raw milk rats (26·6 ml., Table VIII) is obviously fortuitous, but the agreement itself gives a very strong indication that vitamin B_1 was the limiting factor in these two experiments.

It should be added that the protein intake of the rats on raw milk plus the higher level of sugar was less than that of the rats on sterilized milk plus the lower level of sugar and amounted to only 8·4% of the solids against 12·8% (on the basis of 0·57% N in milk and a protein conversion factor of 6·38).

This difference is to a certain extent compensated by the lower biological value of the proteins of sterilized milk (9). The almost identical efficiency of utilization of calories of the two groups (Table IX) shows that the lower protein intake did not handicap the raw-milk rats. In any case a low level of protein intake, while possibly restricting growth, would not affect the caloric intake provided other factors were adequately supplied. In other words if the raw milk rats were getting more of the appetite-controlling factor than the sterilized milk rats (which we know to have been restricted in this respect), they would have consumed more food.

Further support to the suggestion that the difference between the nutritive efficiency of the raw and sterilized milks is due to a difference in their respective contents of vitamin B_1 is given by the performance of the rats which received a basal diet in addition to a fixed limited supply of milk (p. 190). When the diet supplied no additional source of the vitamin B complex a sharp differentiation could be observed between rats on raw and those on sterilized milk. The level of milk intake was still lower than in the experiments just discussed and amounted to only some 16 ml. (Table VIII). In this case the rats on sterilized milk grew very poorly but did not show any specific deficiency symptoms. On the other hand, two out of six rats given sterilized milk plus the higher level of sugar which also took 16 ml. of milk, developed unmistakable symptoms of vitamin B_1 deficiency, but it should be pointed out that the latter animals were lighter at the start of the experiment and thus more likely to show symptoms.

With another basal diet differing from the former only in the inclusion of

[1] In only two cases out of six are the comparisons between litter-mates.

Table IX. *Weights; gains in weight; daily gains in weight; intakes of milk+sugar solution, milk, sugar, total solids and calories and calorie intake/g. gain in weight, for the last twenty days of the experiment, of the rats receiving (a) sterilized milk+15 g. sugar/100 ml., (b) raw milk+30 g. sugar/100 ml.*

Rat no.	Weight (g.) 8 days	Weight (g.) Final	Gain (g.)	Gain/day (g.)	Intake: Milk+sugar solution (ml.)	Intake: Milk (ml.)	Intake: Sugar (g.)	Intake: Total* solids (g.)	Calorie† intake	Calorie intake/g. gain in wt.
				Sterilized milk+first level of sugar (15 g./100 ml.)						
5996[1]	65	144	79	3·95	1122·0	929·0	139·4	263·7	1252·0	15·85
6000	57	113	56	2·80	795·0	722·7	108·5	205·2	973·9	17·39
6004	54	122	68	3·40	866·5	787·7	118·2	223·6	1061·5	15·61
6008	40	92	52	2·60	660·0	600·0	90·0	170·3	808·5	15·55
6011	58	141	83	4·15	1082·0	983·6	147·6	279·2	1325·5	15·97
6016[2]	55	100	45	2·25	827·0	751·8	112·8	213·4	1013·8	22·53
Average	54·8	118·7	63·8	3·19	875·4	795·8	119·4	225·9	1072·5	17·15
				Raw milk+second level of sugar (30 g./100 ml.)						
5997[1]	48	115	67	3·35	657·5	547·9	164·4	236·9	1069·8	15·97
6001	41	111	70	3·50	666·0	555·0	166·5	239·9	1083·6	15·48
6005	40	94	54	2·70	603·0	502·5	150·8	217·3	981·1	18·17
6009	42	89	47	2·35	557·0	464·2	139·3	200·7	906·2	19·28
6014	41	116	75	3·75	708·0	590·0	177·0	255·1	1151·9	15·36
6017[2]	48	94	46	2·30	638·5	532·1	159·6	230·0	1038·8	22·58
Average	43·3	103·8	59·8	2·99	638·3	531·9	159·6	230·0	1038·6	17·81

* The solids in milk are calculated from the data in Table I of the first paper of this series (4) on the assumption that the specific gravity of milk is 1·03.

† The caloric value of milk is calculated from its fat percentage (obtained from data in Table I of the first paper of this series(4)) using the formula of Overman & Sanmann(7).

The caloric value of sugar is taken as 4·1 Calories/g.

[1, 2] denotes litter-mates.

5% brewer's yeast it was not possible to distinguish between raw and sterilized milk, though the level of milk intake was, as before, 16 ml.

Though yeast doubtless supplied various other factors and other members of the vitamin B complex, we feel, in view of the evidence brought forward in the preceding pages, that its value in making good the shortcomings of sterilized milk in the present experiments lay in its vitamin B_1 content and we believe that, at lower levels of intake in experiments of this type, vitamin B_1 is the first limiting factor of sterilized milk.

Quite recently Kohler *et al.* (10) have published a paper criticizing the use of the paired feeding method in experiments involving the exclusive feeding of milk supplemented with minerals and pointing out that in their opinion young rats used for such experiments have to be specially prepared by not allowing them access to the mother's diet. The latter finding was also very kindly communicated earlier to one of us (S. K. K.) by Prof. Hart (11). At that time the present work was almost completed.

We have since carried out experiments in which the methods of the Wisconsin workers were closely followed and were compared with our present technique. The results will be published in detail later but it may be stated here that no revision of the conclusions arrived at in the present series of papers is demanded.

Summary

1. The effect of commercial sterilization on the total nutritive value of milk was studied in five separate experiments.

2. In the first experiment raw and sterilized milks supplemented with minerals were given in equal amounts to pairs of litter-mate male rats. No differences were found in the growth performance of the rats, but the rats on sterilized milk consumed it more readily than their mates on raw milk. This difference was statistically significant.

3. Rats getting limited but equal amounts of milk in addition to a basal diet which supplied only protein, energy and minerals grew better on raw than on sterilized milk.

4. When the basal diet contained in addition 5% brewer's yeast and the milk intakes were the same as in the previous experiment no difference was found between the two groups.

5. Rats receiving raw milk to which 15 g. of cane sugar was added per 100 ml. grew better than rats receiving equal quantities of similarly treated sterilized milk.

6. Rats given raw milk containing 30 g. per 100 ml. of sugar grew as well as the rats on sterilized milk in the preceding experiment, but rats receiving similarly treated sterilized milk took much less milk than the raw milk rats, grew very poorly and some of them developed symptoms of beri-beri.

7. The difference between raw and sterilized milk is discussed and it is concluded that in these experiments on the total nutritive value of milk vitamin B_1 was the first limiting factor of sterilized milk.

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612.392.841:637.133.4

190. THE EFFECT OF COMMERCIAL STERILIZATION ON THE NUTRITIVE VALUE OF MILK

VII. CONCLUSIONS

By S. K. KON

The work carried out by us on the nutritive value of commercially sterilized milk shows definitely that certain factors are injured in the course of the heat treatment. The biological value of the proteins is slightly but unmistakably decreased, most probably because of the partial destruction of one or more essential amino-acids. Of the vitamins, vitamin C is the most markedly affected. It is decreased by half and, in view of the severity of the heat treatment, it is remarkable that the loss is not greater. The anaerobic conditions which exist during the application of the highest sterilizing temperatures may account for the survival of a part of this labile factor. Vitamin B_1 also suffers serious loss, the destruction amounting to 30% of the original value. Neither vitamin A and carotene nor vitamin B_2 (flavin) are affected by the heat treatment. The fate of other components of milk was not studied by us, but experiments on rats on the effect of sterilization on the total nutritive value of milk indicates that vitamin B_1 was the first limiting factor of sterilized milk.

From the nutritional aspect sterilized milk is inferior to raw or to pasteurized milk(1), though it still remains a most valuable foodstuff. Its bacteriological purity and its exceptional keeping quality make it a product particularly attractive to mothers for use in the feeding of infants and children and account for its spread in certain centres(2). It must, however, be clearly understood that sterilized milk is not the equivalent of raw or pasteurized milk and that if it is used to replace them, its shortcomings must be recognized and repaired by the addition of the necessary supplements.

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637.221:532.13

191. THE SPREADING CAPACITY OF BUTTER. I

By G. W. SCOTT BLAIR

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(With 2 Figures)

Davis (1) has recently described experiments on the rheology (flow and deformation properties) of cheese. His rheometer, although primarily designed for cheese, was used by him for butter as well, but, as the outcome of subsequent discussion, it seemed desirable to devise a modification of the method which would be especially suited to butter work. Whereas with cheese elastic moduli are at least as important as viscosities in defining consistency, with butter the elastic properties are much less marked. Their possible practical significance will not be lost sight of, but in this preliminary study only viscous properties will be considered.

Many butters are found to be difficult to spread at the temperature of the British breakfast room, and not a few become too soft to satisfy the public during hot summer weather. Butter makers are shy of attempting to correct at least the former of these faults by modifications in the manufacturing process, because it is felt that such changes may be detrimental to keeping quality. This is particularly true of butter shipped from distant parts of the Empire. It is clearly desirable to ascertain what physical properties of butter are concerned in spreading capacity and to develop a method for measuring them.

Various methods have already been proposed for a direct measure of spreading capacity by imitating the process of spreading and measuring the rate at which this has taken place for a standard load, or alternatively the load required to spread at a given rate. Other empirical methods for measuring hardness of butter have also been described (2,3,4,5). It might at first sight appear that a direct empirical test would be the best way of measuring spreading capacity. It must be remembered, however, that spreading capacity is a psychological composite in which a number of separate physical properties is concerned. The proportion of each factor concerned is not known. The empirical test likewise measures a composite of properties which may or may not be the same as those included in the original conception, but which are most unlikely to feature in the same proportions.

It is therefore better to isolate each recognizable physical property and to measure it in absolute units. If the isolated properties are not constants independent of practical changes in condition, this involves choosing arbitrarily standardized conditions, but even these are better defined than is the conglomeration of properties given by an empirical test.

The nearest approach to a measure of individual physical properties of butter with a view to estimating hardness (apart from Davis' work already quoted) would appear to be that of Hunziker, Mills and Spitzer(6). These authors measured the amount of compression of a cylinder of butter of standard size under a load of 300 g. applied for 20 sec. They did not, however, calculate the viscosity in absolute units, nor did they study the variations of viscosity with stress.

It has been shown for other materials(7) that the latter factor is closely connected with shortness or brittleness, a point already noted by Davis. It seems probable that this, together with "hardness" (resistance to shear) are the two principal factors involved in spreading capacity. In the case of a true fluid, the resistance to shear is measured by viscosity, which is defined as the constant ratio of shearing stress to rate of shear. For materials in which this ratio is not constant, it is nevertheless justifiable to speak of the viscosity for a given stress under standard conditions. Even for true fluids, viscosities must be quoted for some arbitrary temperature, and for a material like butter, a viscosity can be given in absolute units for a given set of stress and deformation conditions. Where these conditions change appreciably during the determination, a mean viscosity can be evaluated. The general theory involved in this method has been fully described by Schofield & Scott Blair(8), and is summarized by Davis(1).

For the purpose of measuring the viscosities of butter at a series of stresses, an apparatus was constructed as shown in Fig. 1. Test pieces of butter are prepared by pressing a cutting ring, 4·0 cm. diam. and 2·0 cm. height (*A*, in figure) into a pat of butter and trimming off the excess with a wire. The apparatus consists in a loading column (*B*) with a wooden base (total weight about 1½ lb.) on which slotted lead weights from 1 lb. up to a total of 32 lb. can be placed. The wooden base has a diameter of 5 cm. The butter sample (*X*) rests on a small ebonite stand, and at the start of the experiment, the loading column, which rests on the supports (*C*) when not in use, is turned and placed on the sample. The column is held vertical by a well-lubricated bearing (*D*) on the top of which is attached a brass ring insulated from the bearing by means of an ebonite ring. Another brass ring is screwed on to the column, and can be adjusted so as to make contact with the first when the column has fallen through a predetermined distance. A compression of 0·79 cm. is chosen since this corresponds to a strain of 0·5,[1] and a disk of height 1·21 cm. (*E*) serves as a convenient way of adjusting the contact rings. These rings are connected to a battery (*F*) and buzzer (*G*), and a stop-watch, started when the load is

[1] Strain in a cylinder is normally expressed as the ratio of deformation (Δl) to original height (l_0). This is only justified if the deformations are small. For big deformations, the correct expression is $\int_l^{l_0} \frac{dl}{l}$ where l is height after compression. This is equal to $\log_e(l_0/l)$ or $2{\cdot}3\log_{10}(l_0/l)$, and $2{\cdot}3\log_{10}\left(\frac{2{\cdot}00}{1{\cdot}21}\right) = 0{\cdot}5$. This expression holds whether the strain is recoverable (elastic) or non-recoverable (flow).

applied, is stopped when the buzzer indicates that the strain of 0·5 has been reached.

This experiment should be done for several different loads, the first load to be tried may be inconveniently large or small, but subsequent loads can be chosen to cover a suitable range. Times of from about 10–500 sec. are con-

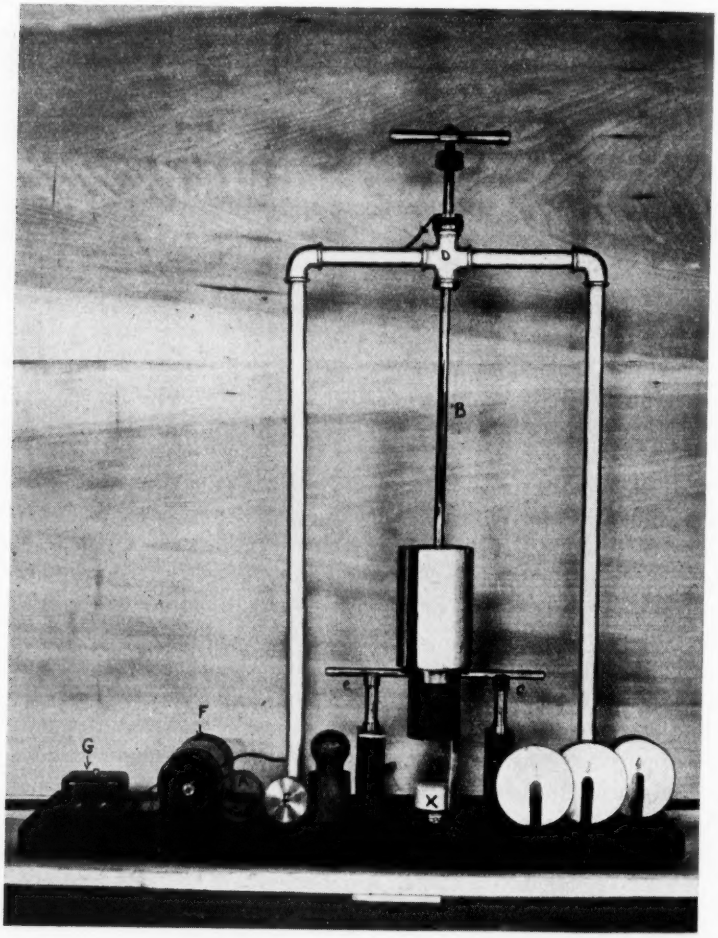

Fig. 1.

venient. For practical purposes, it is sometimes adequate to plot load directly against log t (where t is time of compression in sec.) but the mean viscosities are easily calculated in c.g.s. units by multiplying the load in pounds (W) by t and again by a constant. The log t/load curves are fairly linear.

The constant is evaluated as follows:

It is first necessary to relate W to shearing stress. For materials such as

butter, in which the Poisson ratio hardly differs from 0·5 (*vide* (8)), i.e. which do not change their volume in compression, the shearing stress in a loaded cylinder is equal to one third of the compressing load calculated in dynes/cm.^{2} At the beginning of the loading, the shearing stress, is therefore given by:

$$S_1 = \frac{W \times 454 \times 981}{3 \times \pi \times 2^2} = 11810\ W \text{ dynes/cm.}^2$$

At the beginning of the loading, the stress will have diminished by an amount proportional to the change in area, that is, to the ratio of the heights of the cylinder at the start and at the finish,[1] so that:

$$S_2 = \frac{11810 \times 1{\cdot}21}{2{\cdot}00}\ W = 7150\ W \text{ dynes/cm.}^2$$

The mean stress is therefore given by

$$S = 9480\ W.$$

The mean rate of shear is given by $0{\cdot}5/t$, so that the viscosity in poises = $18960\ Wt$.

It is more convenient to express this in megapoises, and in these units we have

$$\eta = 0{\cdot}01896\ Wt \text{ megapoises.}$$

This viscosity is, of course, a mean for the loading process. The viscosity tends to rise during the loading as the stress falls, and it tends also to rise with compression (work-hardening).

In order to distinguish between the effects of stress and strain on viscosity, a light metal beam was pivoted to the frame of the apparatus, one end of the beam being depressed by the falling loading column, the other end being pointed, and indicating the amount of the fall of the beam on a scale. The scale gave a magnification of 2·45 : 1. A series of cylinders cut from the same butter were compressed at varying loads, the compression/time curves being obtained in each case. The mean stresses and strains corresponding to each pair of readings were calculated, and from these, and the times, viscosities were evaluated. These are given in megapoises in the diagram shown on Fig. 2. The four sets of points correspond to loads of 6·5, 7·5, 8·5 and 9·5 lb. respectively from left to right. It is clear that the viscosity rises enormously with rising strain for any given stress, and, at least for higher strains, falls rapidly with rising stress at constant strain (structural viscosity). It is interesting that at low strains, although the accuracy of the experiment is not adequate to present many data, the structural viscosity is apparently not very great. This experiment was done on a sample of butter which had been recently worked. Preliminary experiments indicate that the capacity to work-harden wears off as the sample gradually recovers from the working, but the whole question requires further investigation.

[1] This assumes that the cylinder is uniformly compressed which is never strictly true. In practice there is a bulging at the centre.

The effect of temperature

The viscosity of butter falls very rapidly with rising temperature and it is therefore essential to keep this factor constant. A constant temperature room is being constructed, but for the preliminary experiments described in this paper, a less convenient thermostatic device (not shown in the figure) was temporarily used. The butter samples were kept in an incubator cooled by brine circulation, and the test piece during compression was contained in a double-walled tin through which cooled brine circulated.[1] In order to make a

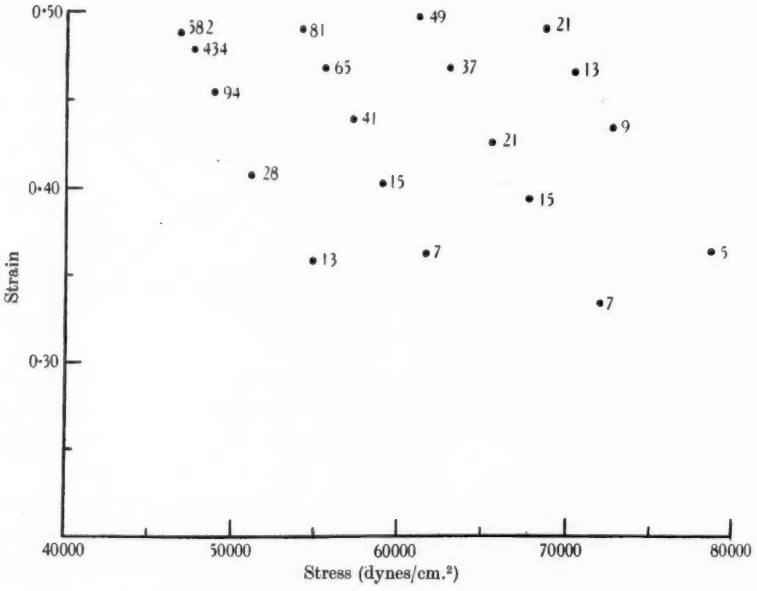

Fig. 2.

close imitation of British breakfast room conditions in winter, it would seem desirable to work at a temperature of not more than 50° F., but at this temperature quite a number of butters, not abnormally unspreadable, crumble and break under load. This crumbling gives bad reproducibility, nor is the above treatment applicable to such behaviour. Crushing strength measurements in all materials are less reproducible than viscosity measurements, and it is better to measure crumbliness indirectly from the rate of fall of viscosity with increasing load. A temperature of 55° F. would seem to be fairly suitable, although samples showing really poor spreadability crumble even at this temperature.

[1] I am indebted to Mr P. Temple for fitting up this arrangement.

Reproducibility

There is no doubt that much greater reproducibility will be possible when the constant temperature room is available, but the viscosity of butter is altered to such an enormous extent by different treatments and samples differ so much *inter se*, that it is no objection to the method that values of t on replicate samples loaded with the same W vary considerably. A detailed study of this question will be made at a later date.

The relationship between rate of shear and shearing stress

Preliminary experiments with butter seem to indicate, that, as with cream (9), over a fair range of stress, the rate of shear varies as a power of the stress. This conclusion is quite empirical, but gives a convenient way of expressing the data, since the log (rate of shear)/log (stress) curves are linear, and their slope gives a measure of the extent of deviation of the material from truly fluid behaviour (structural viscosity). It is probable that this figure is a measure of "crumbliness", and possible that it may also be related to the way in which spreadability varies with changes in temperature. The physics of these phenomena is being investigated and will be discussed elsewhere.

The effect of gentle working on the spreading capacity as measured by the rheometer

A large piece of very hard, unspreadable butter was tested at a number of different loads, and the load required to give a strain of 0·5 in 1 min. (L) determined by interpolation. The sample was then reworked,[1] and tested again at intervals. Later it was reworked a second and a third time, and again tested at intervals. The temperature was 55° F. throughout. Table I gives the results of this experiment, L being quoted in lb.,[2] and η in megapoises.

Table I

Treatment		L	η
Control	... ...	30*	34·1
1 working, rested	1 hr.	16·5	18·8
	3 hr.	17·5	19·9
	20 hr.	23·0	26·2
	44 hr.	26·0	29·6
2 workings, rested	¾ hr.	13·0	14·8
	5 hr.	12·0	13·6
	22 hr.	12·0	13·6
3 workings, rested	2½ hr.	11·0	12·5
	21½ hr.	11·5	13·1
	123 hr.	17·0	19·4

* Samples crumbled somewhat. L should therefore really be somewhat higher for a true flow.

These results show a marked "false body" for butter, a phenomenon already described by Hunziker, Cordes & Nissen (10). After the first working,

[1] I am indebted to Miss D. V. Dearden, Head of the Dairy Department, N.I.R.D., for the reworkings.

[2] The load is given as the weight of the lead disks + 1½ lb. for the loading column.

the viscosity fell by 45%, but gradually recovered, until after two days, the original consistency was nearly re-established. The second working reduced viscosity by 50%, but no recovery took place during 22 hr. The third working reduced by only 8%, and a slow recovery, although not apparent after one day, was evident after 5 days.

Temperature control was not sufficiently accurate to justify conclusions about any changes in the structural viscosity.

Summary

1. The problem of the physical nature of spreading capacity of butter is discussed, and the advantages of methods measuring individual physical properties in absolute units are given.

2. A simple rheometer is described with which the rate of compression of cylinders of butter can be measured. A simple equation for calculating absolute (mean) viscosities from the data obtained with the apparatus is given.

3. Preliminary experiments, in which temperature control was not as exact as might be desired, nevertheless gave an effective measure of hardness. These are quoted to show the applicability of the method, but they also show the extent to which the phenomenon of "false body" occurs when butter is reworked.

4. A preliminary experiment on the work-hardening of butter is described.

The writer wishes to express his best thanks to Dr J. G. Davis for suggestions for the design of the rheometer based on his experience with the model originally designed for cheese.

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192. VOLATILE ACIDS OF CHEESE

I. RETENTIVE POWER OF CHEESE AND ITS CONSTITUENTS

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It is generally accepted that the development of volatile acids is an important factor in determining the flavour of cheese. A study of the volatile acids should therefore serve as a useful adjunct to ordinary chemical analysis in defining the quality of a cheese.

Since the classic studies of Orla-Jensen (1) other investigators have extracted and analysed the volatile acids of many types of cheese, and their findings have formed a basis for theories of the course of the ripening processes. Suzuki *et al.* (2), in their studies of the development of flavour in Cheddar cheese, determined the volatile acids at various stages of ripening and concluded that the flavour was due not only to the free acids themselves, but also to their esters and alcohols. Currie (3) found that caproic, caprylic and capric were the chief volatile acids of Roquefort cheese, and he attributed the characteristic peppery flavour to the accumulation of these acids or their readily hydrolysable salts. Lane (4) has studied the effects of eight strains of mould on the ripening of Iowa Blue cheese. He found that while the types of volatile acid produced were, in general, the same for all strains, the total quantities varied. On the whole, the cheese containing relatively small quantities of volatile acids were usually lacking in flavour. Corminboeuf (5) examined various types of blue veined cheese procurable in Canada and found that the volatile acid content decreased in the order Grove City, Roquefort, Gorgonzola and Stilton, corresponding to a decreasing intensity of flavour. He concluded that the characteristic flavours of these cheeses were intimately connected with the development of caproic, caprylic and capric acids. Lane & Hammer (6), in their studies of the influence of the use of homogenized milk in the manufacture of Iowa Blue cheese, used determination of volatile acids as a means of assessing the value of this preliminary treatment of the milk.

In view of the importance of volatile acids it is desirable that a true estimate of the quantities of the various individual acids present should be readily obtainable. In the various investigations hitherto made the volatile acids have been extracted almost exclusively by means of simple steam distillation of a cheese mush. No standardization has been attempted, and the quantity of cheese taken, the volume of water with which it was mixed, and the quantity of distillate collected have been varied at the will of the investigator. In all cases, however, the cheese mush has been made strongly acid, in order to iberate all volatile acids from their salts.

In earlier investigations simple steam distillation was used in a study of the volatile acids of Stilton and Cheddar cheese (7), and in an attempt to use the distribution of the volatile acids as a means of differentiating between various types of English hard-pressed cheese (8). As work progressed serious defects in the method became apparent. It was suspected that only a portion of the volatile acids was being collected, and if this were so irregularities in the relative proportions distilling over might well lead to erroneous conclusions.

Preliminary tests showed that complete recovery of measured quantities of acids added to the cheese before distillation, was rarely, if ever, attained. It appeared that one or more constituents of the cheese possessed the power of retarding the rate of distillation and in some cases even of preventing complete recovery of the added acid. The fat, for instance, was found to have a marked influence on the rate of distillation of the added acids (9).

A more detailed study was then started of the effect of various constituents of cheese upon the rate of distillation of volatile acids associated with the cheese. In a first series the test substances used, the fat and the protein of a white Stilton, were obtained by repeated washing of the finely minced cheese with water and ether respectively. The results obtained were essentially similar to those of a second series to be described later, but frequent irregularities occurred and were suspected to be due to deficiencies in the apparatus, which had been assembled from ordinary laboratory glassware, and contained a number of rubber bungs and lengths of rubber tubing. It was, therefore, decided to erect a battery of distillation units each consisting entirely of glass and containing only interchangeable parts of standard specification. With this standardized apparatus reproducible and therefore more reliable results could be obtained, and a second series of tests was begun.

For this series the fat and protein from a mature Cheddar cheese were used, and for purposes of comparison samples of the cheese from which these constituents were extracted, washed butterfat made from fresh unripened cream, and rennet-precipitated casein, a commercial product supplied by United Dairies, Ltd., were also examined. The cheese fat was obtained by centrifuging a cheese mush and transferring the fat layer to a separating funnel where it was freed as completely as possible from casein by repeated washings with warm water and was finally filtered to remove all traces of water. The protein was separated by shaking the finely minced cheese with alcohol to remove water and repeatedly with ether to remove fat. Preliminary washing with water was omitted in this case, which probably accounts for the presence of the measurable quantity of volatile acids shown in Table I, for which allowance had to be made in computing the results. Chemical analyses of the test substances gave the following figures:

Cheese fat	Trace of casein.
Butter fat	No casein.
Cheese protein	0·35% fat.
Casein	0·30% fat.

Acetic, propionic, butyric, caproic, caprylic and lauric acids used in these tests were the purest obtainable as supplied by Kahlbaum. The capric acid, procured from America, through Griffin and Tatlock, was the purest available, but bore no analytical guarantee.

Standard ($1/10N$) solutions of the soluble and $1/20N$ solutions of the sodium salt of the insoluble acids were used. Ten ml. of the former and 20 ml. of the latter were added to a mixture of 20 g. of the test substance in about 120 ml. of boiled distilled water, and the whole was well shaken and placed in a cold room overnight. To equalize conditions the requisite quantity of NaOH solution was added to convert the free soluble acids into their sodium salts. The following morning the mixtures were brought to *p*H 2 with 10% H_2SO_4, the volume made up to 200 ml. and steam distillation started. The distillate, five times the volume of the original mixture, i.e. 1000 ml., was collected and titrated in 100 ml. quantities. This progressive method, although liable to cumulative errors of titration, was considered necessary for the plotting of curves. The higher insoluble acids were apt to be deposited in the condenser. They were removed by washing the condenser with alcohol between each fraction for lauric, between alternate fractions for capric and only after the second fractions for caprylic acid. All determinations with the acids were made in duplicate, and controls of the test substances in triplicate, at intervals throughout the period of the test. As far as possible 500 ml. flasks were used for the distillations, but the excessive frothing of the cheese protein, cheese and casein mixtures, necessitated the use of distilling flasks of 2000 ml. capacity. In the first series of tests an attempt had been made to obviate this difficulty of frothing by the addition of liquid paraffin. The results were not wholly satisfactory, and the use of larger flasks seemed preferable.

The results of the second series of tests have been collected in Tables I–VII and the means shown in the form of graphs in Figures 1 and 2.

Table I. *Control distillations of substrates. Mean values*

Times original volume	ml. $N/10$ NaOH required to neutralize					
	Water mean of 3	Cheese mean of 3	Cheese fat mean of 3	Cheese protein mean of 3	Butterfat mean of 3	Casein mean of 2
½	0·05	3·62	0·1	1·4	0·08	0·125
1	0·10	6·15	0·2	2·55	0·16	0·25
1½	0·15	8·0	0·3	3·575	0·23	0·375
2	0·20	9·43	0·4	4·375	0·30	0·5
2½	0·25	10·6	0·5	5·05	0·37	0·625
3	0·30	11·52	0·58	5·5	0·42	0·73
3½	0·35	12·27	0·65	5·775	0·47	0·835
4	0·40	12·88	0·73	6·125	0·52	0·94
4½	0·45	13·45	0·8	6·4	0·56	1·045
5	0·50	14·02	0·87	6·75	0·60	1·15

Table II. *Percentage added acids recovered in fractions from water*

Times original volume	Acetic			Propionic			Butyric			Caproic			Caprylic			Capric			Lauric		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
½	23·75	23·75	23·75	40·2	36·0	38·1	48·3	47·3	47·8	79·0	75·6	77·3	84·0	74·1	79·05	80·3	—	80·3	31·45	16·46	23·95
1	41·2	41·2	41·2	64·8	59·6	62·2	71·6	71·1	71·35	93·25	91·9	92·58	90·7	99·2	94·95	92·7	92·5	92·6	65·35	47·0	56·18
1½	57·0	56·0	56·5	79·9	73·1	76·5	89·0	85·0	87·0	97·1	97·1	97·1	93·4	101·5	97·25	96·4	99·4	97·9	85·2	79·4	82·3
2	71·3	71·3	71·3	88·8	91·9	90·35	97·5	92·0	94·75	99·5	98·6	99·05	95·7	102·4	99·05	99·7	101·2	100·45	94·0	95·8	94·9
2½	82·4	84·0	83·2	97·2	96·6	96·9	102·0	97·6	99·8	102·0	99·5	100·75	97·0	102·4	99·7	—	—	—	98·3	97·3	97·8
3	92·4	92·4	92·4	98·2	99·7	98·95	105·5	100·5	103·0	—	—	—	97·5	102·4	99·95	—	—	—	100·75	98·3	99·58
3½	101·3	102·4	101·85	98·7	101·3	100·0	—	—	—	—	—	—	—	—	—	—	—	—	100·75	99·7	100·23
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4½	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Table III. *Percentage added acids recovered in fractions from cheese*

Times original volume	Acetic			Propionic			Butyric			Caproic			Caprylic			Capric			Lauric		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
½	19·84	10·35	15·1	42·4	27·15	34·78	52·0	48·1	50·05	52·0	66·3	59·15	38·9	26·8	32·85	15·46	9·79	12·63	6·1	3·2	4·65
1	32·75	21·65	27·2	70·5	42·65	56·58	77·6	76·1	76·85	72·7	89·5	81·1	68·7	49·0	58·85	31·75	26·6	29·18	11·13	3·39	7·26
1½	44·35	33·25	38·8	89·0	54·3	71·65	91·6	89·1	90·35	83·3	94·7	89·0	81·8	64·2	73·0	42·35	34·65	38·5	15·0	5·81	10·4
2	55·1	46·6	50·85	100·2	62·9	81·55	96·7	98·7	97·7	86·8	97·3	92·05	88·7	75·2	81·95	54·9	46·2	50·55	13·75	8·42	11·09
2½	62·9	55·0	58·95	104·7	69·0	86·85	98·5	100·5	99·5	87·5	96·5	92·0	90·7	77·7	84·2	58·9	50·8	54·85	15·55	11·13	13·34
3	72·2	63·7	67·95	107·7	71·4	89·55	98·8	103·7	101·75	89·7	96·5	93·1	91·5	80·3	85·9	65·6	57·7	61·65	17·22	15·8	16·51
3½	81·1	73·7	77·4	106·6	73·5	90·05	98·3	106·8	102·55	90·25	95·5	92·88	91·5	81·5	86·5	70·2	61·5	65·85	19·16	18·86	19·01
4	86·3	82·0	84·15	105·7	74·4	90·05	98·3	111·2	104·75	90·1	94·9	92·5	89·2	82·4	85·8	75·2	67·1	71·15	21·5	21·98	21·74
4½	89·3	88·2	88·75	104·2	74·2	89·2	96·5	112·25	104·35	88·5	93·7	91·1	89·4	81·7	85·5	77·8	70·25	74·03	22·75	25·65	24·2
5	91·2	92·2	91·7	101·8	72·9	87·35	94·3	112·0	103·15	88·3	91·7	90·0	88·8	79·8	84·3	80·8	72·5	76·65	24·0	28·37	26·19

Table IV. *Percentage added acid recovered in fractions from cheese fat*

Times original volume	Acetic			Propionic			Butyric			Caproic			Caprylic			Capric			Lauric		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
½	29·55	32·2	30·88	42·6	38·95	40·78	48·3	49·75	49·03	56·0	50·3	53·15	20·65	21·1	20·88	5·23	5·06	5·5	2·91	3·39	3·15
1	51·7	52·8	52·25	68·4	61·6	65·0	72·7	79·1	75·9	83·8	72·8	83·3	37·25	42·2	39·73	9·5	8·75	9·13	3·87	5·32	4·6
1½	69·2	67·6	68·4	82·0	76·8	79·4	87·1	93·0	90·05	93·7	86·2	89·95	52·1	56·15	54·13	13·76	12·42	13·09	5·32	6·3	5·81
2	82·4	79·7	81·55	90·5	86·4	88·45	94·0	100·5	97·25	97·6	92·8	95·2	67·4	65·15	66·23	17·58	17·1	17·34	6·29	7·75	7·02
2½	93·5	89·8	91·65	95·3	93·7	94·5	97·5	106·0	101·75	99·6	96·7	98·15	78·2	72·3	75·25	20·9	19·8	20·35	6·77	8·72	7·75
3	101·5	96·9	99·7	97·6	98·1	97·85	100·2	109·1	104·65	100·3	98·3	99·3	85·6	78·8	82·2	24·9	24·1	24·5	9·88	9·88	9·88
3½	109·7	104·5	107·1	99·5	101·5	100·5	—	—	—	101·0	99·5	100·25	89·8	84·5	87·15	27·5	27·6	27·55	11·13	10·65	10·89
4	—	—	—	100·2	104·3	102·25	—	—	—	—	—	—	92·7	87·7	90·23	30·1	30·55	30·33	12·28	11·31	11·8
4½	—	—	—	—	—	—	—	—	—	—	—	—	94·3	89·7	92·0	32·25	33·15	32·7	13·06	12·6	12·83
5	—	—	—	—	—	—	—	—	—	—	—	—	96·8	91·4	94·0	35·45	36·65	36·05	13·84	13·36	13·6

Table V. *Percentage added acid recovered in fractions from butterfat*

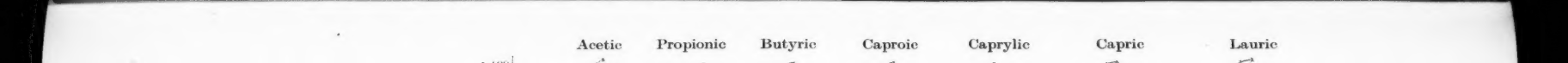
Times original volume	Acetic			Propionic			Butyric			Caproic			Caprylic			Capric			Lauric		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
½	29·25	26·6	27·93	31·27	32·85	32·56	54·4	46·0	50·2	60·0	56·7	58·35	16·36	24·9	20·63	5·71	4·94	5·33	2·13	4·55	3·34
1	50·6	46·3	48·45	50·4	54·2	52·3	78·0	73·5	75·75	83·6	81·25	82·43	29·55	48·0	38·78	15·52	10·36	12·94	4·26	6·68	5·47
1½	66·2	62·5	64·35	67·6	67·6	67·6	90·2	89·3	89·75	91·6	92·0	91·8	46·9	63·1	55·0	21·8	14·43	18·3	5·04	8·42	6·73
2	78·7	77·1	77·9	79·5	76·35	77·93	96·0	97·5	96·75	97·0	97·6	97·3	62·0	72·8	67·4	24·85	22·8	23·83	6·29	10·64	8·47
2½	89·6	89·5	89·55	87·7	82·9	85·3	98·8	100·61	102·45	98·5	99·8	99·15	72·1	79·4	75·75	31·1	26·85	28·98	7·55	12·4	9·98
3	97·5	99·0	98·25	93·4	87·2	90·3	—	—	—	98·5	100·7	99·6	79·8	84·3	82·05	38·58	32·55	35·57	9·49	13·84	11·67
3½	104·3	106·9	105·6	96·1	90·8	93·45	—	—	—	—	—	—	84·7	88·3	86·5	43·6	36·85	40·23	10·46	15·3	12·88
4	—	—	—	98·7	93·0	95·85	—	—	—	—	—	—	89·7	91·9	90·8	50·0	40·2	45·1	11·9	18·68	15·29
4½	—	—	—	100·4	94·7	97·55	—	—	—	—	—	—	92·4	94·25	93·33	55·15	43·1	49·13	13·46	20·25	16·86
5	—	—	—	101·55	95·8	98·68	—	—	—	—	—	—	94·3	96·15	95·23	62·2	46·1	54·15	15·0	22·75	18·88

Table VI. *Percentage added acid recovered in fractions from cheese protein*

Times original volume	Acetic			Propionic			Butyric			Caproic			Caprylic			Capric			Lauric		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
½	25·2	24·4	24·8	39·8	41·2	40·5	59·7	57·75	58·73	76·9	73·6	75·25	87·5	89·0	88·25	22·1	72·2	—	22·16	28·4	25·28
1	44·4	40·8	42·6	64·75	67·6	66·18	82·1	78·65	80·38	92·6	87·4	90·0	98·4	96·1	97·25	92·2	106·8	101·5	53·7	47·0	50·35
1½	56·1	55·35	55·73	77·0	79·3	78·15	90·7	87·8	89·25	96·2	89·0	92·6	96·8	96·3	96·55	97·7	105·6	—	70·65	64·05	67·35
2	63·4	65·75	64·58	83·5	84·4	83·95	91·75	90·8	91·28	94·2	88·0	91·1	90·5	94·5	92·5	99·6	105·6	102·6	80·9	75·5	78·2
2½	69·2	70·8	70·0	90·25	89·8	90·03	91·8	90·05	90·93	—	—	—	—	—	—	—	—	—	88·2	84·7	86·45
3	75·6	76·8	76·2	93·5	93·5	93·5	94·0	91·5	92·75	—	—	—	—	—	—	—	—	—	93·1	91·7	92·4
3½	81·0	81·35	81·18	97·8	96·5	97·15	95·7	94·25	94·98	—	—	—	—	—	—	—	—	—	99·7	95·5	97·6
4	83·7	85·4	84·55	98·4	98·4	98·4	97·7	95·2	96·45	—	—	—	—	—	—	—	—	—	99·7	95·0	97·35
4½	87·5	88·0	87·75	99·0	99·0	99·0	98·0	95·05	96·53	—	—	—	—	—	—	—	—	—	99·7	98·4	99·05
5	91·1	90·75	90·93	100·0	100·0	100·0	97·0	94·55	95·78	—	—	—	—	—	—	—	—	—	—	—	—

Table VII. *Percentage added acid recovered in fractions from casein*

Times original volume	Acetic			Propionic			Butyric			Caproic			Caprylic			Capric			Lauric		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
½	28·15	26·15	27·15	24·45	40·0	37·23	60·4	59·9	60·15	74·8	77·7	76·25	87·7	86·5	87·1	44·4	72·5	58·45	21·4	36·1	28·75
1	44·8	46·0	45·4	62·5	63·4	62·95	93·5	96·1	94·8	92·9	95·25	94·08	98·35	97·9	98·13	113·8	116·4	115·1	48·35	49·65	49·0
1½	62·2	59·75	60·98	78·9	78·0	78·45	110·6	109·2	109·9	100·2	101·2	100·7	101·2	—	101·2	—	—	—	68·9	68·5	68·70
2	72·5	70·0	71·25	89·8	85·65	87·73	—	—	—	—	—	—	—	—	—	—	—	—	77·2	83·8	80·05
2½	79·8	78·2	79·0	96·0	93·2	94·6	—	—	—	—	—	—	—	—	—	—	—	—	79·6	88·4	84·0
3	87·0	87·75	87·38	101·1	97·0	99·5	—	—	—	—	—	—	—	—	—	—	—	—	81·75	91·5	86·63
3½	93·7	91·3	92·5	102·8	99·7	101·25	—	—	—	—	—	—	—	—	—	—	—	—	83·4	92·8	88·1
4	96·5	97·7	97·1	103·4	101·5	102·45	—	—	—	—	—	—	—	—	—	—	—	—	83·4	95·4	89·4
4½	100·0	101·9	100·95	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—



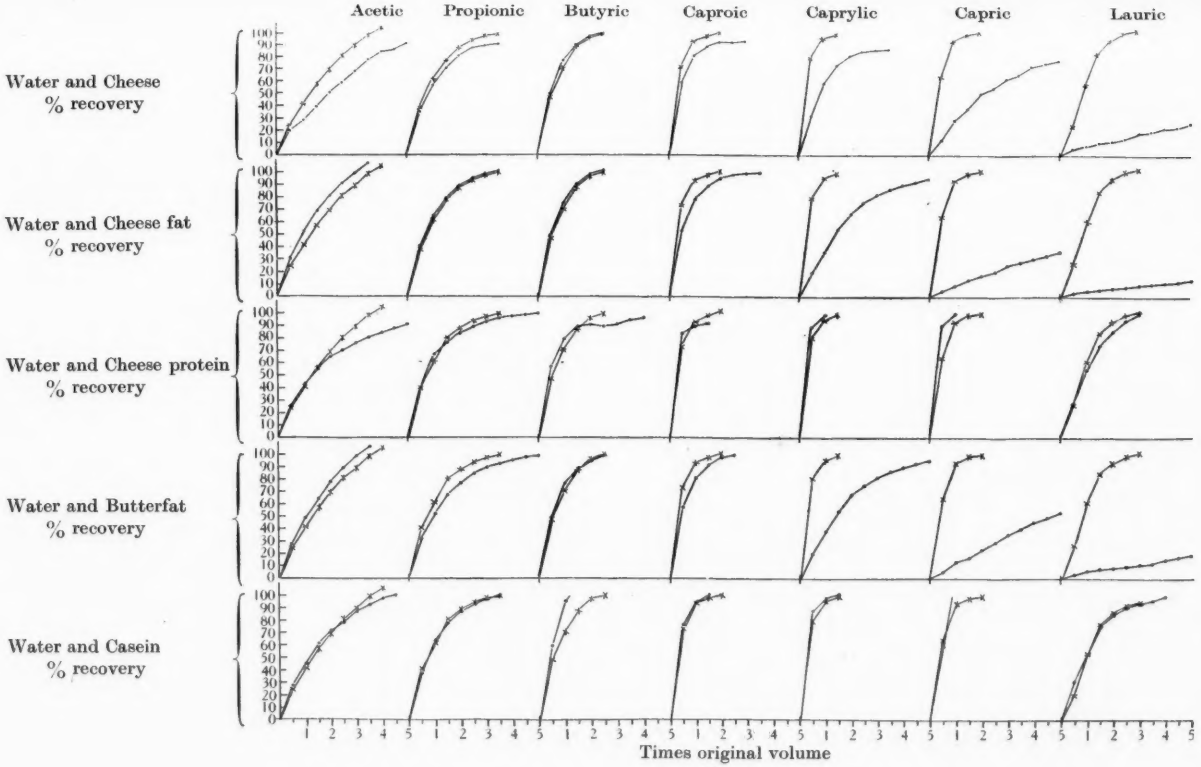


Fig. 1.

×—×—× Acid recovered from water. ·—·—· Acid recovered from test substance.

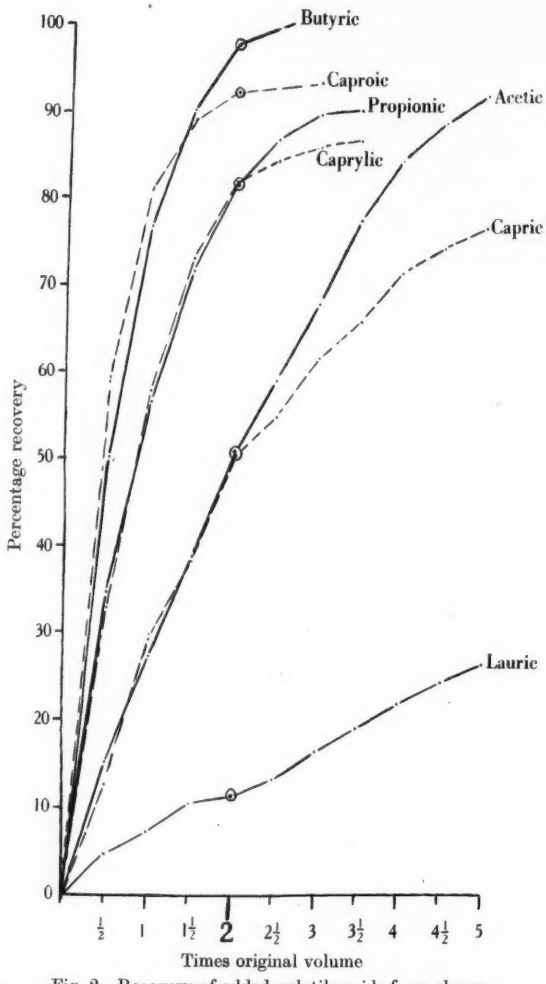


Fig. 2. Recovery of added volatile acids from cheese.

Discussion of results

The rates of distillation from *water* closely followed those given by Dyer (10). Variations may probably be attributed to differences in apparatus and in working technique. There was always complete recovery of the added acid within the limits, "5 times original volume", of the distillation.

With *cheese* there was a distinct retardation of the rate of distillation, scarcely significant for butyric acid, and only slight for caproic, but for all others well-marked. Moreover, it appeared that complete recovery was attainable only for butyric acid however great the volume of distillate collected.

For caprylic, caproic and probably propionic acids, a maximum recovery of less than 100% was reached in a distillate of not more than "4 times the volume". For acetic, capric and lauric acids the full quantities had not been recovered at "5 times the volume", but there was no evidence that the maximum had been reached at this stage. The results are suggestive of a permanent retention of a small percentage of acid by the cheese, in which case complete extraction of volatile acids could never be attained by simple steam distillation. In this connexion it should be emphasized that in these tests a solution of the sodium salt of the acid was left in contact with the cheese for a few hours only, and the opportunity for adsorption of or combination with the acid was much less than might be expected when small quantities of acid are being continuously produced throughout a long ripening period. The amount of non-distillable volatile acid in a normal cheese may be greater than is suggested by these figures. The pairing of the curves in Fig. 2 indicates a similarity between the initial rates of distillation of a higher insoluble and its corresponding lower soluble acid. In each pair the curves practically coincide until "twice the volume" has been distilled, after which there is an increasingly great divergence. It is probable that a distillate of "twice the volume" would give a fair conception of the relative proportions of the various volatile acids present, although the maximum distillation had not been reached.

Both *cheese fat* and *butterfat* exert a definite influence on the distillation rate of the acids. The water-insoluble fat-soluble acids were retarded, the extent of the retardation increasing with increasing solubility in fat and decreasing solubility in water. Thus, for caproic acid practically the whole of the acid has been recovered in "3 times the volume" whereas for lauric acid less than 20% has distilled over in "5 times the volume". There was no significant retardation for butyric acid, and for the water soluble acids, propionic and acetic, the rate of distillation was accelerated by the presence of the fat. The influence of the cheese fat was in all cases somewhat greater than that of butterfat, indicating that the composition of the fat is a factor that must be considered.

The influence of the *cheese protein* and the *casein* was less marked than that of the fat and also of a reverse order, inducing an acceleration in the rate of distillation of the higher insoluble acids with the exception of lauric. Complete recovery of each of the added acids was possible from the casein mixtures, but the action of the cheese protein was very similar to that observed for cheese itself. Unlike the casein, the cheese protein appeared to hold a small percentage of certain acids in such a form that it was not recoverable by simple steam distillation.

A salient feature of these results is the steady change in the accelerating or retarding action of each test substance on the rate of distillation throughout the series of acids. In the tests for which the data are given, the rate of distillation of butyric acid was scarcely affected by the presence of either of the substances, and the effect on caproic acid was also very slight. The action increased steadily from this point up and down the series of acids, towards the

lower and higher members respectively. Some important members of the series had been omitted from these tests but had been included in earlier investigations(11), from the results of which it may be inferred that the critical point in the series is at isovaleric acid.

In general, these studies suggest that the accelerating or retarding action of certain constituents of cheese is of such a nature that the relative proportions of the volatile acids in fractions collected during the early stages of distillation bear little relationship to their original distribution in the cheese. The discrepancies will be most marked for the highest and lowest members of the series. This difficulty can be evaded, as far as the fat at least is concerned, by continuing the distillation long enough to overcome its action. Even so it appears that one or more of the constituents of the cheese, probably breakdown products of the casein, still hold an undetermined percentage of some of the acids so strongly that they cannot be removed by simple steam distillation. Other means of extraction are necessary for their recovery from the cheese. This point has only been touched upon and needs further study, but it is possible that it offers at least a partial explanation of the difficulties experienced in attempting to make satisfactory studies of the volatile acids in cheese.

Summary

1. Direct steam distillation of cheese does not give a true figure for the volatile acids present in cheese.

2. The fat portion of a cheese has a retarding effect on the distillation of higher volatile fatty acids, although it is possible that a complete recovery of the acid may be effected from the fat if the distillation were to be continued for a sufficiently long period.

3. Cheese protein is capable of a permanent retention of a part of some of the acids present, it being impossible to extract these portions by continued distillation.

4. If direct steam distillation of a cheese is to be carried out the results seem to indicate that by collecting only "twice the original volume" a more accurate picture is gained of the distribution of the acids present in the cheese than would be obtained by distilling to "5 times original volume", since the retention factor of the protein becomes more significant after "twice the volume" (Fig. 2).

The authors wish to acknowledge the help received in this investigation from Mr J. Z. Wolf and Mr A. G. House.

APPENDIX

The standard apparatus designed to eliminate errors due to irregularities in size and construction was made by Messrs Standley, Belcher and Mason, Ltd., of Birmingham, who have also supplied the line drawing and specification in Fig. 3.

We wish here to acknowledge the help and advice given by Mr H. G. Forster of this firm in developing the original design and the various modifications introduced later.

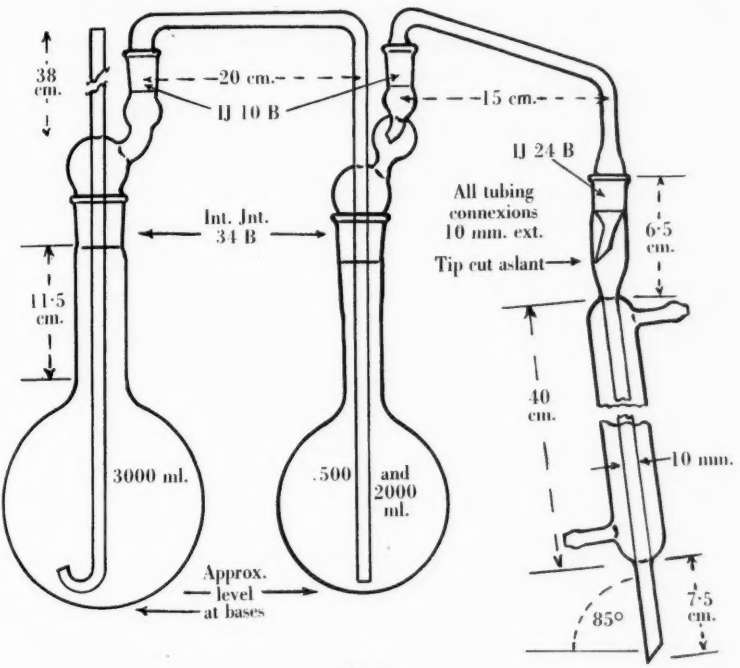


Fig. 3.

The construction of each unit is clear from the figure. When the apparatus is in use the boiling flask is filled with distilled water to which a few pieces of pumice are added. The bend at the bottom of the safety tube prevents bubbles from the pumice escaping into the tube when the flask is boiling vigorously. These bubbles cause an overflow of water and might crack the flask and also cause a suck back from the distilling flask owing to a sudden fall of pressure of the steam.

The water is allowed to boil to get rid of CO_2 before placing an empty flask in position. The apparatus is washed out with steam, approximately 100–200 ml. of water being collected before replacing the empty flask with one

containing the material to be distilled. The exchange is made by lowering the washing flask until free from the steam tube and then inserting the other. After sufficient distillate has been collected the distilling flask is replaced with an empty flask and the washing process repeated.

When working with acids which are liable to accumulate in the condenser tube it is sometimes necessary to wash them out in order to estimate the quantity distilled in each fraction. This is done by momentarily dropping the condenser and washing through with neutral alcohol from a wash bottle. The loss sustained by this procedure is, as far as can be ascertained, practically negligible.

It is unnecessary to dismantle the apparatus to refill the boiling flask as this may be done by pumping water down the safety tube by means of a blow ball. In cases when there is insufficient water to complete a distillation, water may be added slowly through the safety tube without impairing the pressure of steam.

It is possible with this apparatus to carry out several consecutive distillations working continuously. This means an appreciable saving of time when numerous distillations are necessary.

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193. VOLATILE ACIDS OF CHEESE

II. METHODS OF EXTRACTION

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Studies in the ripening of cheese have indicated that important changes in the volatile acid content and distribution occur during the ripening period. These changes are not the same for all types of cheese and appear to be of particular interest for Stilton cheese into which an active lipolytic agent is introduced with the growth of the mould. The accumulated evidence of the extensive nature of these changes (1) justified an attempt to study them in greater detail, but the work was suspended in order to examine the possibilities of developing more exact methods than those in general use for the extraction and differentiation of the volatile acids of cheese. The knowledge of the strong lipolytic power of *Penicillium roqueforti* presages the development of higher volatile fatty acids in the last stages of ripening of blue-veined cheese; but these have been shown to be the acids most seriously affected by the retentive power of cheese when an attempt is made to extract them by simple steam distillation (2). If a true idea of the distribution of the acids is to be attained there must be complete extraction for each acid, or at least such constant proportions must be recoverable that accurate estimations can be made. It is doubtful whether such perfection can ever be realized by simple steam distillation of a cheese mush. A more satisfactory procedure would appear to be preliminary extraction by means of suitable solvents; steam distillation of these solutions would not then be subject to the disturbing influence of various constituents of the cheese.

The direct use of dilute alkali is precluded because of the physical changes induced in the body of the cheese, making the withdrawal of the solution impracticable. The fact that the higher acids are soluble in fat suggested the use of ether, a solvent for acids and fat; the acids could be removed from this solution by washing with dilute alkali. Preliminary tests involving ether extraction of weighed quantities of finely minced cheese showed that values could be obtained which were slightly higher than those for simple steam distillation in the case of English hard-pressed cheese, and appreciably higher in the case of blue-veined varieties. The increase seemed to be due to the extraction of larger quantities of the higher fatty acids, measurable amounts of which were sometimes deposited in the condenser. Erratic results in early experiments appeared to be due to emulsification of the fat during the washing out of the acids from the ether solution. Cheese fat seemed to be readily emulsified if subjected to too vigorous shaking in the presence of dilute alkali. Hydrolysis of the fat was then demonstrable, hence it is important that the washing with dilute alkali should be with gentle shaking only.

Experience had also suggested water as an effective solvent for many of the volatile acids and their salts. Various methods of extraction were therefore tried using either water or ether as the chief solvent, followed by a secondary extraction with the alternative solvent. Simple steam distillation of a cheese mush was used for comparison. Both ripe Cheddar and well-blued Stilton cheese were tested in this way.

Two essentials required of the method finally selected would be (*a*) simplicity of technique, allowing its use for routine examination of reasonably large numbers of cheese, (*b*) rapid execution, avoiding further changes in the volatile acid distribution by continuation of the action of enzymes already present in the cheese.

Tests were carried out according to the following plan:

A quarter of a cheese was used in each case; the peripheral layer was removed to a depth of $\frac{1}{2}$–$\frac{3}{4}$ in. and the remaining cheese macerated. An ordinary household meat-mincing machine was found to be most effective for Stilton cheese owing to its soft texture, but for the relatively hard Cheddar a toothed mill was preferred. The minced cheese was thoroughly mixed before sampling.

All steam distillations were made on cheese mush adjusted to *p*H 2 with H_2SO_4 using thymol blue as indicator. The liquid in the distilling flask was kept at constant volume and the distillate collected always measured five times this original volume (usually expressed as "5 times the volume"). All distilled water used for washing the cheese and for adjusting the volume before distillation was boiled to expel CO_2 and cooled.

The first series of experiments was carried out on a well-blued Stilton cheese having a *p*H of 6·8 determined electrometrically.

(1) *Direct steam distillation*

Two 20 g. samples of the cheese were acidified, made up to 100 ml. and steam distilled (500 ml. distillate collected). Four other 20 g. samples were spread in a thin layer along the walls of porcelain dishes and dried in desiccators under reduced pressure for 3 days, two being placed at 4° C. and two at 37° C. After 3 days these were acidified, made up to 100 ml. and steam distilled (500 ml. distillate collected).

(2) *Water washing followed by ether extraction*

Water solution. Eight 20 g. samples of cheese were placed in separate centrifuge jars and mixed with 100 ml. of boiled distilled water at 40–45° C.

Four of these were shaken *by hand* for $\frac{1}{2}$ hr. after which they were centrifuged and chilled in a refrigerator at 4° C. The hardened fat layer was then transferred by means of a spatula to about 50 ml. of ethyl ether and the aqueous layer in the centrifuge jars poured into distilling flasks. The protein residues at the bottom of the jars were subjected to two further successive washings with 100 ml. of distilled water. The jars were well shaken,

centrifuged, chilled and any fat removed to the ether as before. The combined water washings (300 ml.) were acidified and steam distilled (1500 ml. distillate collected).

The other four samples were subjected to 3 hr. shaking on an *automatic rotary shaker*; by this means most of the fat was churned into balls incorporating some casein and most of the mould mycelium. These balls of fat were transferred to another set of centrifuge jars each containing 100 ml. warm distilled water, well shaken by hand to separate the casein and mould from the fat, and finally centrifuged. The jars, from which the fat balls had been removed, were centrifuged in order to deposit suspended protein and bring any remaining free fat to the surface. After centrifuging, both sets were chilled and the fat transferred to ether as before. The aqueous layers were combined in distilling flasks and the protein residues mixed and subjected to a further washing with 100 ml. of distilled water. The three combined washings were then acidified and steam distilled (1500 ml. distillate collected).

Ether-fat solutions. The *ether-fat solutions* from both types of shaking were placed in separating funnels and each one washed with six successive 50 ml. quantities of $N/10$ NaOH, care being taken not to emulsify the whole by too vigorous shaking during the first two or three washings. The washings were freed from ether, acidified and steam distilled (1500 ml. distillate collected).

Ether solutions. Of the *protein residues* in the centrifuge jars, two from each of the hand shaken and automatically shaken groups were mixed with washed sand, brought to *p*H 2 and extracted with ether in a Soxhlet apparatus for 36 hr. The ether extracts were then washed in separating funnels with four successive 50 ml. quantities of $N/10$ NaOH. These washings were freed from ether, acidified and steam distilled (1000 ml. distillate collected).

The remaining two residues from each set were acidified, made up to 100 ml. and steam distilled (500 ml. distillate collected).

(3) *Ether extraction followed by washing with water*

Ether solutions. Eight 20 g. samples of cheese were mixed with washed sand and extracted with ether in Soxhlets at the natural *p*H of the cheese. Four of these extractions were stopped at the end of 2 hr., the other four were continued for 36 hr. Four other 20 gr. samples were acidified to *p*H 2 mixed with sand and extracted in a Soxhlet with ether for 36 hr. Another eight 20 g. samples were spread in a thin layer over the sides of porcelain dishes and dried in desiccators under reduced pressure, four at 4° C. for 3 days and four at 37° C. for 3 days. After 3 days' drying all samples were mixed with sand for extraction. A duplicate pair from each temperature was left at the natural *p*H of the cheese, and extracted for 2 hr. only; the remaining pairs were acidified to *p*H 2 and extracted for 36 hr.

All the *ether extracts* from this series were washed with six successive 50 ml. quantities of $N/10$ NaOH: the ether was removed from the washings which were then acidified and steam distilled (1500 ml. distillate collected).

Water solution. The *protein residues* of the samples which were not subjected to preliminary drying were removed from the thimbles; two were acidified, made up to 100 ml. and steam distilled (500 ml. distillate collected). The remaining two samples were subjected to two successive washings with 100 ml. of distilled water, and centrifuged after each washing; the washings were acidified and steam distilled (1000 ml. distillate being collected).

The residues of those samples which had been dried in the desiccator before extraction with ether, were all acidified, made up to 100 ml. and steam distilled (500 ml. distillate being collected).

As a result of experience gained during the extraction of the volatile acids from Stilton cheese, a few modifications were introduced into the scheme when it was applied to Cheddar cheese. With the exception of the differences noted below the former procedure was followed in detail.

(1) *Direct steam distillation*

Preliminary drying of the samples was omitted from the ether extraction methods, hence there was no necessity for comparative distillation of dried samples. Only the duplicate samples of fresh cheese were submitted to direct steam distillation.

(2) *Water washing followed by ether extraction*

No changes.

(3) *Ether extraction followed by washing with water*

The drying of samples was omitted, since this appeared to lead to a loss of acids. Variations were made, however, in the treatment of the cheese prior to extraction. Eight samples were adjusted to *p*H 2, eight to *p*H 7, and mixed with sand; a third group of eight samples was mixed with anhydrous Na_2SO_4—8 g. Na_2SO_4 to 20 g. cheese. Four samples from each group were extracted with ether in a Soxhlet apparatus for 2 hr., the remainder for 36 hr. The subsequent procedure was that used in the experiments with Stilton cheese. The results are given in Table I.

Discussion of results

A striking feature of these figures is the marked difference between the values for the total volatile acids as determined by direct steam distillation of the cheese itself and those obtained by distillation of the water and ether extracts. The latter were always much higher than the former. The water soluble acids and salts that could be extracted from the untreated cheese simply by shaking with water were in themselves in excess of the total acids extracted by direct steam distillation. In addition to these, an appreciable quantity could be recovered from the fat by washing the ether-fat solution with dilute alkali, and a still further quantity from the protein residues by extracting with ether. In this connexion attention may be drawn to the fact

that steam distillation of the protein residues yielded a lower figure than ether extraction, thus providing another illustration of the retaining power of cheese protein noted in Part I (3).

The variation in the method of shaking gave slight differences in the distribution of the acids amongst the three fractions, but the total value was essentially the same. The higher values for the water solution when the cheese was automatically shaken for 3 hr. were obviously due to more efficient washing of the cheese, since the values for the acids extracted with ether from the protein residues were correspondingly low. There was very little variation in the values for the fat-soluble fraction. There is no evidence of lipolysis of the fat during the prolonged shaking such as was feared might happen in the presence of the mould when Stilton cheese was being treated. Both methods of shaking appeared to be equally satisfactory; the áutomatic shaking demands less physical effort, which is a recommendation.

Contrary to expectation the various methods in which extraction with ether represented the chief operation always gave significantly low figures. This may have been due to loss of volatile acids by evaporation from the mouth of the Soxhlet apparatus, although the temperature of the water bath was held at 40–50° C. and a rapid current of cold water was passed through the condensers. In this connexion it is interesting to note that while a 2 hr. extraction gave higher values for the acidified than for the neutral cheese this difference was very much less marked after 36 hr. extraction. On the whole the figures for the ether extraction (36 hr.) method were inconsistent and comparatively low.

In the case of Stilton cheese the protein residues from the 36 hr. extraction gave higher yields of volatile acids when subjected to steam distillation than when extracted with water. This difference was not observed for Cheddar cheese, and is quite the reverse of that noted for final treatment of the protein residues and in the water extraction method. The significance of this point has not yet been studied; it is probable that it is correlated with the relative proportions of the soluble and insoluble volatile fatty acids in the two types of cheese.

Drying the cheese for 3 days before extracting with ether seemed an unnecessary complication of the method. The results were erratic, pointing to a loss of volatile acids by evaporation under reduced pressure. Mixing the finely divided fresh cheese with sand appeared to be quite adequate to ensure the penetration of the ether.

The water extraction method appears to offer a suitable means of estimating the volatile acids in cheese. In the series of tests recorded in this paper the results were in fair agreement and reproducible. Moreover, although higher than any of the other figures, they were obtained without heating the cheese or altering its natural *p*H. It is proposed to adopt this as a routine method, and further work is therefore being directed towards the elaboration of detail.

This series of experiments has shown that by water extraction of the cheese and alkaline extraction of the fat, followed by ether extraction of the residues,

a value for the volatile acids may be obtained which is about $4\frac{1}{2}$ times as high as that from direct steam distillation in the case of Stilton cheese and about twice as high in the case of Cheddar. Other estimations have confirmed these findings and it may be assumed that the difference is usually of this order. This raises the question of the validity of the conclusions (frequently reported in the literature) that have been drawn from previous estimations of the volatile acids based on direct steam distillation of cheese.

SUMMARY

An extraction method for estimating volatile acids in cheese has been described. The values obtained were considerably higher than those given by direct steam distillation.

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194. THE CALCIUM AND PHOSPHORUS CONTENTS OF SOME TYPES OF BRITISH CHEESE AT VARIOUS STAGES DURING MANUFACTURE AND RIPENING

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Since 1920(1) when Droop Richmond published tables giving the proximate composition of various cheeses, very large numbers of analyses of British cheese have been made, but although different types may have been studied, these have generally been made under entirely different conditions. The number of published analyses of the various types of British cheese manufactured from the same bulk milk are very few, although MacDowall & Dolby(2) have recently reported extensive analyses of cheese of the Cheddar type.

In 1935 and 1936, a comprehensive study of the manufacture and ripening of British cheese was begun and the opportunity to determine the distribution and quantities of certain mineral constituents (ash, calcium and phosphorus) was taken, the results obtained forming the subject of this paper. The nitrogen distributions, the volatile acid determinations and detailed bacteriological examinations carried out at the same time by other workers will be reported later. In 1935 from the same batch of raw milk (*a*) Cheddar, (*b*) Cheshire, (*c*) Leicester and (*d*) Lancashire cheese were made. In 1936 the experiment was repeated but Stilton replaced the Lancashire type. The differences in the four types of hard pressed cheese result from greater or lesser variations in technique during the process of making and the mature cheese have, of course, widely different characteristics. It is not possible to elaborate the cheese-making details here but to facilitate the appreciation of the determinations which have been made, the essential differences are given.

Cheddar cheese

Acidity is allowed to develop before rennet is added and moisture is expelled by cutting the curd small and scalding at a high temperature. After pitching, acidity increases, the curd mellows and a close texture is obtained, by milling in large pieces. Considerable pressure is applied at once to the warm curd. A close texture and firm body result.

Cheshire cheese

Only slight acidity is developed before renneting and acidity slowly develops in the later processes. The curd is milled small at comparatively low acidity when the moisture content is still high. After 20 hr. pressure is applied

with the temperature remaining at 70° F. The curd granules do not completely coalesce and the texture is granular with a firm body.

Leicester cheese

Acidity is kept comparatively low, the curd cut small and a low scalding temperature used. At the low acidity prior to pressing the curd is resilient and when pressed the pieces preserve their identity.

Lancashire cheese

The milk is renneted with very little acidity and as in Cheshire cheese this develops slowly later. The curd is cut to medium size without heating, pressure being applied to expel moisture. A part of the curd is held for 24 hr. before milling. By mixing old and new curd the acidity is adjusted and, as drainage from the two curds after mixing is not the same, the granules, which are pressed after 18 hr., do not coalesce, the texture being loose with a soft body.

Stilton cheese

Quite sweet milk is renneted and coagulation is slow. Similarly, the drainage of whey is slow, without heat or pressure. This is secured by transferring the curd, by ladling, into cloths which are tightened at intervals. There is therefore considerable "bathing" of the curd in whey—a point of considerable importance in the light of the data given later. After hooping, drainage continues without pressure for 7 days. The general texture is close with intervening spaces and the body fairly firm.

McCammon *et al.* (3) determined the calcium and phosphorus in a number of Cottage (soft) cheese made under laboratory conditions and found insignificant differences between the rennet and acid types of this variety. Cheddar cheese was, however, found to contain about ten times as much calcium as these soft cheeses.

Wode (4) also showed that as acidity at renneting time increased the total calcium of cheese decreased. Van Slyke (5) also emphasizes this point. Guittonneau and Chevalier (6) showed that the amount of calcium and phosphorus found in whey is related to the presence or absence of a lactic fermentation. They claim that the value of the calcium/phosphorus ratio is always 1·50 for cheese with little acid fermentation prior to renneting and 0·5 for acid coagulated cheese.

McDowall & Dolby (2), working apparently with pasteurized milk, record steady increases in the concentration of calcium and phosphorus in the whey taken at various stages in the process up to salting, the calcium rising more rapidly than the phosphorus. They also confirm earlier work upon the effect of increases in acid development before renneting upon the loss of calcium in the whey.

EXPERIMENTAL

First Series, 1935

To avoid recording details which are not strictly relevant cheese-making records are omitted but the analyses and the calculations based upon them are given in Table I. (The methods used for analyses were published in 1932(7).)

Table I

	Cheddar	Cheshire	Leicester	Lancashire	
Original milk:					
Time	8.30 a.m.	8.30 a.m.	8.30 a.m.	8.30 a.m.	
Titratable acidity	0·18	0·18	0·18	0·18	
Ash %	0·75	0·75	0·75	0·75	
Calcium %	0·122	0·122	0·122	0·122	
Phosphorus %	0·098	0·098	0·098	0·098	
Whey at cutting:				1st curd	2nd curd
Time	10.25 a.m.	10.30 a.m.	10.12 a.m.	6. xi. 35	7. xi. 35
Titratable acidity	0·13	0·13	0·12	—	—
Ash %	—*	0·56	0·57	0·56	—
Calcium %	—*	0·043	0·044	0·043	(0·045)†
Phosphorus %	—*	0·053	0·049	0·049	(0·092)†
Whey at drawing off:					
Time	12.25 p.m.	12.00 noon	11.30 a.m.	—	—
Titratable acidity	0·175	0·15	0·145	—	—
Ash %	0·58	0·58	0·59	(0·59)†	—
Calcium %	0·049	0·049	0·047	0·051	0·058
Phosphorus %	0·046	0·045	0·051	0·051	0·054
				Both curds	
Time of grinding curd	3.10 p.m.	2.50 p.m.	1.25 p.m.	—	
Whey from press 24 hr. later:					
Titratable acidity	0·90	0·57	0·45	—	
Ash %	13·62	6·6	7·52	—	
Calcium %	0·235	0·267	0·233	0·43	
Phosphorus %	0·137	0·151	0·134	0·20	
Cheese at 139 days:					
Ash %	3·75	3·59	3·86	3·93	
Calcium %	0·80	0·69	0·76	0·66	
Phosphorus %	0·51	0·47	0·49	0·44	

* Lost sample.

† Bracketed figures are results of one determination only.

In both series an effort was made to collect all the whey throughout manufacture. In practice, the difficulties were great but the volumes measured were probably sufficiently accurate for the amounts of ash, calcium and phosphorus remaining in the curd (calculated from those lost in whey) to be representative and comparable between the different types. In the second series, analyses of the curd at successive stages were also made.

The figures for the percentages of ash, calcium and phosphorus of the original milk remaining in the cheese after 24 hr. in press are given in Table II. The amounts lost during the 24 hr. and the calcium phosphorus ratio in the cheese four months later are also given.

It appears that, if the degree of acidity developed at the time of renneting is the determining factor, the differences between the four types of cheese in

Table II. *Percentage of ash, calcium and phosphorus of the original milk remaining in cheese after* 24 *hr. in press*

Constituent	Cheddar	Cheshire	Leicester	Lancashire
Ash	51·2	55·7	53·2	—
Calcium	65·5	65·4	68·0	65·8
Phosphorus	58·1	51·2	55·0	60·0
Percentage of original substances lost during 24 hr. pressure				
Ash	5·7	8·4	4·5	—
Calcium	0·5	1·9	0·8	1·1
Phosphorus	0·5	1·5	0·6	0·7
Calcium : phosphorus ratio in cheese 4 months old				
	1·57	1·47	1·55	1·50

this respect are not great enough to cause material differences in the amounts of calcium and phosphorus retained in the green curds. Thus, in spite of the fact that Cheshire, Leicester and Lancashire are all renneted at lower acidities than Cheddar, the amounts of ash, calcium and phosphorus retained differ only slightly but probably significantly.

In Cheddar, Cheshire and Leicester 51–56% of the original mineral matter is retained; in all four types 65–68% of the calcium and 51–60% of the phosphorus. These figures are of the order of those reported by McDowall & Dolby (2).

It appears that in cheese of this kind, practically the whole of the mineral loss takes place at the drawing of the whey. The high percentage of ash lost in the whey during 24 hr. pressing is no doubt due to the salt added at time of hooping and although the percentage of calcium is high, the actual quantity is not great as the volume is small.

The calcium phosphorus ratios in the ripe cheese are of the order of that given by Guittonneau & Chevalier (6) for pressed cheese, viz. 1 : 1·5.

Second series, 1936

In the second series Stilton was substituted for Lancashire, and analyses of whey and of curd were made at intervals throughout the manufacture, and of cheese during the ripening period.

The figures for the *p*H values, titratable acidity (milk, starter and whey), the percentages of ash, calcium and phosphorus and the calcium phosphorus ratio are shown in Table III for milk and starter, Table IV for whey, and Table V for curd and cheese.

Table III. *Milk and starter*

	Original milk	Starter
*p*H	6·69	—
Titratable acidity	0·175	8·42
Ash %	0·77	0·78
Calcium %	0·124	0·127
Phosphorus %	0·098	0·095
Calcium/phosphorus	1·265	1·34

Table IV. *Analyses of whey during manufacture of cheese*

	Cheddar	Cheshire	Leicester	Stilton
Time of adding starter 22. ix. 36	8.42 a.m.	8.45 a.m.	8.40 a.m.	9.20 a.m.
Time of adding rennet	9.46 a.m.	9.40 a.m.	9.34 a.m.	9.40 a.m.
*p*H of milk	6·55	6·64	6·57	6·69
Titratable acidity of milk	0·21	0·205	0·20	—
Cutting (or ladling) of curd:				
Time	10.5 a.m.	10.27 a.m.	10.30 a.m.	10.53 a.m.
*p*H	6·43	6·57	6·45	6·52
Titratable acidity of curd	0·15	0·15	0·15	0·11
Ash %	0·57	0·565	0·57	0·56
Calcium %	0·049	0·0495	0·050	0·047
Phosphorus %	0·050	0·050	0·050	0·049
Calcium/phosphorus	0·94	0·99	1·00	0·96
Drawing off of whey:				
Time	11.35 a.m.	11.50 a.m.	12.25 p.m.	3.0 p.m.
*p*H	6·13	6·10	6·22	6·02
Titratable acidity	0·315	0·295	—	0·40
Ash %	0·58	0·59	0·59	0·625
Calcium %	0·054	0·054	0·054	0·067
Phosphorus %	0·053	0·054	0·048	0·058
Calcium/phosphorus	1·02	1·00	1·125	1·14
Whey from press, 23. ix. 36 (Cheddar and Leicester):		Overnight drainings		Overnight drainings
Time	8.30 a.m.	8.30 a.m.	8.30 a.m.	9.0 a.m.
*p*H	5·52	—	5·38	4·98
Ash %	11·63	6·64	5·08	1·14
Calcium %	0·26	0·28	0·215	0·26
Phosphorus %	0·16	0·17	0·13	0·12
Calcium/phosphorus	1·625	1·65	1·65	2·17
Whey from press (Cheshire):				At putting away
Time	—	11.10 a.m.	—	11.0 a.m.
*p*H	—	5·52	—	5·17
Titratable acidity	—	1·44	—	—
Ash %	—	5·13	—	6·54
Calcium %	—	0·375	—	0·235
Phosphorus %	—	0·213	—	0·14
Calcium/phosphorus	—	1·785	—	1·64
Whey 24 hr. after putting away (Stilton) 24. ix. 36:				
*p*H	—	—	—	4·79
Ash %	—	—	—	5·11
Calcium %	—	—	—	0·26
Phosphorus %	—	—	—	0·15
Calcium/phosphorus	—	—	—	1·73

In considering the figures in Table IV, the similarity in the composition of the whey in all four types of cheese at the time of cutting the curd is noticeable, but at the time of draining about one (Cheddar) to four (Stilton) hours later, small differences are found, the ash, calcium and phosphorus being slightly higher in the Stilton than in either of the other three wheys. Twenty-four hours later, marked differences in the percentage of ash are shown, and slight ones in the calcium and phosphorus; during this 24 hr. pressure has been applied to the Cheddar and Leicester cheese, but not to the Cheshire or Stilton. The whey from the Cheshire press after 24 hr. shows rather higher

calcium and phosphorus percentage than from the Cheddar or Leicester, but from the Stilton (no pressure except weight of curd) while the ash percentage is lower, the percentage values of calcium and phosphorus are almost identical with those of Cheddar on the previous day. Although these differences between the whey mineral content of Stilton and the hard pressed cheese appear to be slight, they are reflected to a somewhat surprising degree in the composition of the ripe cheese, as is shown by the figures in Table V.

Figures for curd analysis given in Table V show that at the time of drawing off whey, ash, calcium and phosphorus percentages are highest in Cheddar and lowest in Stilton. This is still the case for calcium and phosphorus, 3 days, 4 months, and 8 months after manufacture, although the Leicester ash is very slightly the highest at 3 days. The marked similarity of the three types of hard pressed cheese and the marked dissimilarity of Stilton are noticeable.

The ash, calcium and phosphorus content of the ripe cheese, compared with that of the milk used in their manufacture is given in Table VI.

It will be observed that in some cases, slightly higher values are given for the ripened cheese than for the unripe curd. In the hard pressed varieties two cheese of each type were made; one was kept for 4 and the other for 8 months; the weight of the one which was analysed at 4 months could, of course, only be estimated at the later date, and this probably accounts for the small discrepancies. Seven Stilton cheese were made and kept for lengths of time varying from 3 weeks to 8 months, necessitating similar estimation of the total weights of cheese at the end of the experiment.

The values for ash in later estimations include the ash from the added salt, the ashing being carried out at low temperature to avoid volatilization. The small discrepancies due to these conditions are not material to the conclusions to be drawn from the analyses.

It is seen that the percentages of the mineral matter of the original milk remaining in the hard pressed cheese are very similar; the Cheddar showing slightly highest and Leicester slightly lowest values, but the Stilton ash is only half, calcium about one-ninth and phosphorus less than half of the values for the hard pressed cheese.

For comparison, several Stilton cheese bought in the market were analysed. As no details of manufacture are known it is not possible to calculate the percentage of the original mineral matter remaining, but the figures for the ripe cheese were:

Type of cheese	Ash %	Calcium %	Phosphorus %	Calcium/ phosphorus
Manufacturer No. 1, Blue unpricked Stilton	2·995	0·258	0·340	0·76
Manufacturer No. 2, Blue much-pricked Stilton	3·373	0·228	0·265	0·86
Manufacturer No. 3, Blue slightly pricked Stilton	3·63	0·207	0·247	0·83

It therefore seems clear that the very low calcium content of Stilton cheese found in these experiments holds generally. This loss during manufacture may

Table V. *Analysis of curd during manufacture and cheese during ripening*

	Cheddar	Cheshire	Leicester	Stilton
Time of renneting 22. ix. 36	9.46 a.m.	9.40 a.m.	9.34 a.m.	9.40 a.m.
Curd at cutting (Stilton-ladled):				
Time	10.6 a.m.	10.27 am.	10.30 a.m.	10.53 a.m.
*p*H	6·44	6·38	6·52	6·53
Ash %	0·79	0·76	0·76	0·77
Calcium %	0·131	0·121	0·119	(0·118)*
Phosphorus %	0·103	0·094	0·094	0·110
Calcium/phosphorus	1·27	1·29	1·28	1·20
At drawing off of whey:				
Time	11.35 a.m.	11.50 a.m.	12.25 p.m.	3.0 p.m.
*p*H	5·88	5·86	5·85	5·49
Ash %	2·16	1·78	1·94	1·12
Calcium %	0·64	0·51	0·57	0·25
Phosphorus %	0·43	0·36	0·39	0·22
Calcium/phosphorus	1·49	1·42	1·46	1·14
Before grinding and salting:				At putting away 23. ix. 36
Time	3.15 p.m.	1.20 p.m.	1.00 p.m.	11.0 a.m.
*p*H	5·54	5·49	5·50	4·79
Ash %	(2·22)	2·04	2·08	1·06
Calcium %	(0·68)	0·60	(0·61)	0·22
Phosphorus %	(0·56)	0·42	(0·41)	0·24
Calcium/phosphorus	(1·21)	1·43	1·49	0·92
Curd after salting:				
Time	3.28 p.m.	1.30 p.m.	1.20 p.m.	11.0 a.m.
*p*H	5·49	5·6	5·7	5·2
Curd at 3 days, 25. ix. 36:				26. ix. 36
*p*H	5·46	5·29	5·32	4·59
Ash %	3·54	3·28	3·69	2·09
Calcium %	0·70	0·64	0·65	0·16
Phosphorus %	0·50	0·44	0·44	0·29
Calcium/phosphorus	1·40	1·45	1·48	0·55
Curd at 7 days, *p*H	5·34	5·55	5·46	4·78
Curd at 22 days, *p*H	5·34	5·05	5·26	4·95
Curd at 63 days, *p*H	5·35	5·42	5·35	4·88
Curd at 65 days:				
Ash %	—	—	—	2·60
Calcium %	—	—	—	0·11
Phosphorus %	—	—	—	0·234
Calcium/phosphorus	—	—	—	0·46
Cheese at 4 months:				
*p*H	5·10	5·19	5·00	4·67
Ash %	4·00	3·43	3·82	2·29
Calcium %	0·71	0·66	0·66	0·093
Phosphorus %	0·495	0·47	0·45	0·266
Calcium/phosphorus	1·43	1·40	1·47	0·35
Cheese at 6 months:				
*p*H	—	—	—	6·08
Ash %	—	—	—	2·98
Calcium %	—	—	—	0·112
Phosphorus %	—	—	—	0·253
Calcium/phosphorus	—	—	—	0·44
Cheese at 8 months:				
*p*H	5·16	5·19	5·17	7·18
Ash %	4·06	3·78	4·05	2·82
Calcium %	0·75	0·675	0·70	0·107
Phosphorus %	0·51	0·47	0·49	0·268
Calcium/phosphorus	1·47	1·44	1·43	0·41

* Bracketed figures are results of one determination only.

Table VI. *Weights of ash, calcium and phosphorus present in four types of cheese at various stages of manufacture and ripening*

Stage of manufacture	Wt. of cheese lb.	Cheddar						Cheshire					
		Ash		Calcium		Phosphorus		Ash		Calcium		Phosphorus	
		Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese
Original milk	{8·24	—	—	—	—	—	—	—	—	—	—	—	—
Starter	{8·8	6·41	—	1·02	—	0·815	—	6·40	—	1·03	—	0·814	—
Curd before salting	90·8	2·02	31·51	0·62	60·78	0·51	62·58	2·03	31·72	0·60	58·19	0·42	51·60
Salt added	1·875	—	—	—	—	—	—	—	—	—	—	—	—
After 3 days	86·13	3·05	36·79	0·60	58·82	0·43	52·76	2·87	33·84	0·56	54·37	0·38	46·68
After 65 days	—	—	—	—	—	—	—	—	—	—	—	—	—
After 4 months	81·84	3·27	39·45	0·58	56·86	0·41	50·31	2·82	33·25	0·54	52·43	0·38	46·68
After 6 months	—	—	—	—	—	—	—	—	—	—	—	—	—
After 8 months	79·06	3·20	38·60	0·59	57·84	0·40	49·08	78·74	2·97	35·02	0·53	51·46	0·37	45·45

Stage of manufacture	Wt. of cheese lb.	Leicester						Stilton					
		Ash		Calcium		Phosphorus		Ash		Calcium		Phosphorus	
		Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese	Wt. in lb.	% left in cheese
Original milk	{8·24	—	—	1·02	—	0·81	—	{9·27	—	1·15	—	0·91	—
Starter	{3·125	6·37	—	—	—	—	—	—	—	—	—	—	—
Curd before salting	93·54	1·95	30·61	0·57	55·34	0·38	47·28	168·0	1·78	24·93	0·37	32·17	0·40	43·96
Salt added	1·62	—	—	—	—	—	—	3·5	—	—	—	—	—
After 3 days	85·80	3·17	39·67	0·56	54·37	0·38	46·54	130·0	2·72	25·56	0·21	18·26	0·38	41·76
After 65 days	—	—	—	—	—	—	—	93·2	2·42	22·74	0·10	8·69	0·22	24·17
After 4 months	76·83	2·93	36·67	0·51	49·51	0·35	42·72	90·2	2·06	19·36	0·084	7·30	0·24	26·37
After 6 months	—	—	—	—	—	—	—	80·47	2·39	22·46	0·09	7·83	0·20	21·98
After 8 months	72·74	2·94	36·79	0·51	49·51	0·36	43·95	71·37	2·01	18·89	0·08	6·61	0·19	20·88

be attributed to the fact that considerable acidity is allowed to develop in the curd after ladling and that the curd is bathed in its own whey.

This very low calcium content of Stilton cheese may or may not be significant from the nutritional standpoint, but it is a fact that the hard pressed varieties retain much more of the calcium of the original milk. These differences in the mineral content probably play a part in determining the flavour and other characteristics of the cheese.

SUMMARY

The loss of calcium and phosphorus at various stages in the manufacture and ripening of British hard pressed cheese (Cheddar, Cheshire, Leicester and Lancashire) has been followed. As all the cheese were made from portions of the same bulk milk the results are comparable.

It has been shown that there is remarkably little difference in the values of the hard pressed varieties, but that Stilton contains much less calcium and considerably less phosphorus than any of the other varieties examined.

My thanks are due to Miss D. V. Dearden for the records of the cheese manufacture and of their individual characteristics; to Mr J. Harrison for the determination of the *p*H values in the second series, and to my assistant, H. Hallett, for much help in carrying out the actual analyses.

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BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION D. THE NUTRITIONAL VALUE OF MILK AND MILK PRODUCTS

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INTRODUCTION

THIS review covers the two years from the beginning of 1936 to the beginning of 1938. So many papers have been published that it was possible to deal only with selected subjects and many valuable papers had to be left unnoticed. For the same reason it has been impossible to deal with milks other than that of the cow. As before, most papers have been read in the original, and those known only from an abstract are marked with an asterisk in the bibliography. An increasing number of important papers is being published in journals accessible only with difficulty. It would facilitate the task of the reviewer if authors would send him reprints of their papers.

GENERAL

Maynard's book on animal nutrition(1) contains an up-to-date chapter on the nutritive value of milk. Three reports(2,3,4) give general information on the nutritive value of milk and milk products. It is regrettable that in the last report the references to the original literature have been discontinued. The various reports and summaries mentioned in the section dealing with human requirements should also be consulted. Various nutritional aspects of English milk are dealt with in Bulletin 31, Ministry of Agriculture and Fisheries(5).

COW'S MILK

I. Nutritional value as estimated in laboratory and field experiments

A. *Raw milk*

(*a*) *General.*

Experiments involving the exclusive feeding to rats of milk supplemented with minerals, were described in the previous review(6). Agreement as to the value of this technique in assessing the nutritive properties of milk has not yet been reached. Kohler *et al.*(7,8) have continued their investigations into the differences in growth-promoting value between winter and summer milk and have found that the addition of small quantities of fresh grass or of grass juice to winter milk raises its growth-promoting properties to the summer level. The improved growth of the rats on the supplemented milk was due to increased appetite and not to better assimilation. The authors conclude, without adducing any experimental proof, that the factor present in fresh grass and grass juice is responsible for the difference between winter and summer milk on the assumption that it is transmitted by the cow from the grass into the milk. The addition of other substances to milk, viz. dried oat grass, liver extract and rice bran also markedly increased the growth rate. Kohler *et al.* state that young rats used for such experiments must be specially prepared by denying them access to the mother's diet. Krauss(9, 10), using *ad libitum* and equalized methods of feeding, was unable to confirm these results, whether rats were specially prepared or not. In experiments involving equalized feeding, Henry *et al.*(11) found, on the whole, no differences in the appetite of pairs of littermate rats receiving simultaneously milk either produced on pasture or "winter" milk. The milks were fed alone, and also in another experiment, as additions to a basal diet. Peterson *et al.*(12) repeated, by the *ad libitum* milk feeding technique, their earlier comparisons (already reported in(6)) of milk produced on A.I.V. silage with winter milk. This time the difference in favour of the silage was only very small and as no details are given it is not possible to say whether it was at all significant. Krauss & Washburn(13) likewise obtained in paired feeding and *ad libitum* trials only very slightly better responses with A.I.V. milk.

Mueller & Ritchie(14) added varying percentages of cocoa to mineralized milk and found that more than 10% affected adversely the growth of rats.

Channon & Channon(15) and Channon & Doran(16) have investigated the reproduction of rats on a diet of raw milk supplemented with minerals and with a biscuit made from white flour and water. In both experiments fertility was good and lactation, which failed completely in the first, was highly successful in the second. The authors are at a loss to explain such marked difference in behaviour of rats under apparently identical treatments.

(*b*) *Vitamins.*

(i) *General.* General information on vitamins present in milk, including the author's original work, is given in Neuweiler's booklet(17). A very useful compilation

of references and up-to-date tables will be found in Daniel & Munsell's *Vitamin Content of Foods* (18). Krauss (19) has reviewed the literature, mostly American, on milk vitamins. The presence in milk of the extrinsic factor of Castle and of a new essential factor has been reported (20, 21, 22).

(ii) *Vitamin A*. The difficulties attaching to the physical estimation of carotene in butter have been mentioned in the previous review (6). The situation has now been simplified as it appears (23) that of the α and β isomerides of carotene only the latter is present in butter. The presence of large and variable amounts of the former described in (6) was due to a technical artefact. The determination of carotene or colour in butter and milk is discussed in papers (24,25,26) and the determination of vitamin A in paper (27).

The very large difference in the vitamin A activity of butters as measured in Denmark and in this country was commented on in the last review (6). Coward (28) has since carried out estimations on Danish butters and on butters purchased in the open market (mostly Empire blended) and obtained an average value of 13·2 I.U. per g. for the former and 35·1 I.U. per g. for the latter. It appears that at least part of the difference may be attributed to an actual difference in potency.

Experiments on the addition of carotene to winter butter to improve its colour and enhance its vitamin A activity have been reported from the United States (29). Though such butter has the depth of colour characteristic of a summer sample its vitamin A activity is obviously less.

The possibilities of adding carotene concentrates to margarine are reported from Russia (30,31), while a very interesting discussion on the problem of compulsory addition of vitamins to margarine has recently taken place in Denmark (32,33,34,35,36,37,38). Much useful information concerning the vitamin A (and D) content of butter will be found there also. The addition of vitamins to margarine is also discussed by Scheunert (39).

The question of the relative biological efficiency of vitamin A and carotene has not yet been definitely settled. The work of Fraps *et al.* (40) confirms the findings, reported in the previous review (6), that weight for weight the vitamin A of butter is more potent than the carotene. The work of Guilbert *et al.* (41), which shows that the quantity of carotene needed to cover the minimum vitamin A requirements of several species is five times greater than that of the corresponding amount of vitamin A, seems to point in the same direction, and is supported in this respect by the findings of De (42) and the earlier ones of Davies & Moore (43). Kon & Gillam (44) have also repeated and confirmed their original findings. On the other hand, Leuschen *et al.* (45) obtained quite good agreement between the vitamin A activity of butter measured biologically and that calculated from physical measurements of vitamin A and carotene. Hauge *et al.* (46,47) have investigated further the factor in soya beans which has a depressing action on the vitamin A content of the butter, and have concluded that the factor is heat-stable, insoluble in alcohol and present in the soya bean itself, soya-bean oil and soya-bean cake. Active charcoal apparently removes a large part of the factor from soya-bean oil.

The interest in various methods of increasing the vitamin A and carotene content of milk and butter continues unabated. A.I.V. silage and molasses silage made mostly from alfalfa but also from other succulent foods have again commanded a good deal of attention (12,40,48,13,49,50,51,52). Further studies of artificially dried grass have also been carried out (51,52,53), and isolated investigations have dealt with the effect of green rye (54), hay, grass silage, hay and silage, and pasture (55,56) and with the effect of graded low intakes of carotene from an unspecified source (57). The effect of hegari fodder and cotton-seed cake (58) has also been investigated. The effects of additions of cod and shark liver oils and of carrots have also been examined (51). It is generally

agreed that A.I.V. silage is a very valuable fodder, though it would appear from paper (12) that as far as the carotene and vitamin A content of the milk is concerned it is less efficient than equivalent quantities of the same fodder in the fresh state, while in paper (52) evidence of lower availability of the carotene of A.I.V. silage as compared with artificially dried grass will also be found. Unfortunately in this work the initial carotene contents of the milks of the experimental groups were widely different. Calculations of carotene balances on A.I.V. fodder seem to be complicated by the apparent increase in the carotene content after acid treatment (13,50). Difficulties in getting the cows to consume enough of the silage are reported by Petersen *et al.* (49).

The efficiency of utilization of carotene from all sources is quite low, the recovery in butter ranging from 0·24 % for large intakes (54) to 2·5 % for smaller intakes (40), the latter figure being corrected for the possible transfer of vitamin A from the body stores of the cow to the butter. Paper (54) confirms the views summarized in the previous review (6), that the vitamin A and carotene content of butter cannot be raised indefinitely by increasing the carotene content of the fodder, but that "ceiling" values are reached.

The vitamin A activity of the butters is still not uniformly expressed in international units and comparisons are complicated by the fact that even when such units are used the values are sometimes measured by biological methods and sometimes arrived at by calculations using various factors. It is interesting to note that the highest values reported for butters produced in Denmark on A.I.V. silage or artificially dried grass do not rise above 20 I.U. per g. (51) while values five times as high are reported for grass silage butters from America (55). It should be noted that values as low as 8 I.U. per g. have been reported from America for Jersey butter produced on unspecified feeding (45).

Loy *et al.* (59) state that after changes in the carotene content of the diet the vitamin A and carotene of butter reaches an equilibrium value in about 11 days, and suggest that such short periods can be used in studying the value of various fodders as a source of carotene and vitamin A in milk. Though this is probably true in many cases, papers (40) and (60) show that much longer periods may be necessary before equilibrium is reached. This is also the experience of the reviewer (61), especially when the change is from a higher to a lower carotene intake.

Shaw *et al.* (62) have compared in biological tests the vitamin A potencies of the milk of groups of ten cows of each of the following breeds: Holstein, Jersey, Guernsey, Ayrshire and Brown Swiss, when maintained under identical conditions of feeding. They found that the activity was proportional to the fat content of the milks, but that there was no appreciable difference in the vitamin A activity of the milk fat of the different breeds.

Gillam *et al.* (63) have carried out similar observations on groups of two cows of the Guernsey, Friesian, Ayrshire and Shorthorn breeds by spectrophotometric measurements and by summation of the carotene and vitamin A values. It was found that the vitamin A activity of the butters decreased in the above order of breeds. The authors point out, however, that their calculations do not take into consideration possible differences in biological activity between the carotene and vitamin A of butter. Curves giving the seasonal fluctuation over 2 years of carotene and vitamin A in butter produced by Shorthorn cows under South of England conditions are given in (5) and results of numerous biological tests of butters produced in different countries at various seasons are given in paper (64). Vitameter and Lovibond tintometer measurements are given by Rietti (65) for butters from the Argentine provinces of Buenos Aires, Santa Fé and Cordova, where cattle are kept throughout the year on evergreen pasture. Little difference was found between winter and summer samples. The author erroneously attributes the "blue value" obtained with the antimony

trichloride reagent to the carotene of butter. De Haas & Meulemans (66) have studied the vitamin A content of cow's milk in Batavia.

An exhaustive study of the content and stability of vitamin A in ghee has been carried out by Banerjee and his colleagues (67,68,69,70,71,72). Vitamin A and carotene values for colostral fats are given in papers (45) and (63). The very high vitamin A values quoted by Scheunert (73) for milk are probably due to a mistake, as they do not tally with normal values he quotes simultaneously for butter.

(iii) *The vitamin B complex.* The essential vitamin nature of free lactoflavin is now well recognized. According to a startling recent report from the laboratories of Karrer and of Verzár (74) the phosphoric acid ester of lactoflavin functions as a distinct vitamin and in rats cures the cutaneous lesions characteristic of vitamin B_6 deficiency, upon which free lactoflavin is without effect. Such a profound biological difference between substances chemically so alike is striking, especially as Rudy (75) has shown that free lactoflavin can be phosphorylated by the action of intestinal phosphatase.

Methods based on the fluorescence of lactoflavin and of thiochrome, a derivative of vitamin B_1, in ultra-violet light have been applied to the determination of these vitamins in various products (76,77) and these methods, suitably modified, can be applied to milk probably with satisfactory accuracy (78,79,80,81,82,83). A test using the growth of the micro-organism *Phycomyces Blakesleanus* has also been suggested for the estimation of vitamin B_1 and has been applied to milk (84) but the results yielded so far for milk in the reviewer's laboratory have not been very encouraging (85).

According to fluorometric measurements cow's milk contains some 20 I.U. of vitamin B_1 and 100–150γ of lactoflavin per 100 ml. (78,81,82). The former figure agrees satisfactorily with biological measurements (5,86). Colostrum contains about three times as much lactoflavin as later milk and there is more of the factor in milk of the Channel Island breeds than in that of other breeds (83). Lindholm's (87) figure for Jersey milk, 780γ per 100 ml. of milk obtained by a biological "increase in weight" method, is so high that it suggests the necessity for further investigation of the method before its specificity is accepted.

The Bourquin-Sherman biological test for vitamin B_2 (88) is said to measure lactoflavin only (89). Another method for the estimation of this substance is suggested by Ansbacher *et al.* (90).

György (91) describes tests for lactoflavin and vitamin B_6 in milk.

(iv) *Vitamin C.* Several relatively simple chemical tests for vitamin C in milk are now in general use and a great many papers have been published on the subject.

Whitnah & Riddell have continued their careful work (92,93,94). They reported (92) an average value of 25·7 mg. of vitamin C per 100 ml. for 1275 samples of milk taken directly from the pail shortly after milking. Such samples could be kept for 24 hr. at 40° F. without appreciable loss. Sixty-eight samples after straining and cooling over well-tinned copper gave a lower value, 21·6 mg. %. With large groups of cows a study of the various factors affecting the level of vitamin C in milk (93) showed that evening milk contains some 10% more vitamin C than morning milk. They also noticed small seasonal variations connected rather with climatic than with nutritional conditions. Cows in the first month of lactation were found to secrete milk about 10% lower in vitamin C than in later months. Feeding of large quantities of vitamin C in green rye did not raise the vitamin C content of the milk, though it increased markedly the excretion in the urine.

Further estimations of the vitamin C content of commercial milk have been reported from the United States (95), Germany (96,97,98), Switzerland (17,99), Sweden (100) (biologically), Norway (101), Estonia (102), Finland (103), England (104), Italy (105), the Far East (106) (by the biological method) and (107) and the tropics (108). The technique

used was not in all cases satisfactory, and in some no account was taken of the reversibly oxidized ascorbic acid. Part at least of the variations can be ascribed to these causes. Exposure of the samples to light before titration was also responsible for some of the low figures reported. It is now generally agreed that the vitamin C content of milk is independent of the ration of the cow, and the statement of Rasmussen *et al.*(95) that "after the first 2 months of lactation, the ascorbic acid content of milk is apparently dependent solely upon the ascorbic acid content of the diet of the cow" is surprising, especially as it does not seem to rest on experimental evidence, as, throughout the experiment, the diet of the cows remained unchanged. Kon & Watson(109) have continued the work on the destructive effect of light. They found that the normal healthy cow secretes only the reduced form of ascorbic acid. Visible light of short wave-length (red light is almost without effect) causes a reversible oxidation most probably to dehydroascorbic acid, which spontaneously decomposes still further, even in darkness, and in the absence of oxygen. Paper(110) lends independent support to this view. A pint bottle of milk exposed on the doorstep for half-an-hour and then kept for 1 hour in darkness loses fully half of its original antiscorbutic properties. It is interesting to note that Doan & Myers(111), in studying the reduction of methylene blue by milk in sunlight, found blue cellophane wrappers more protective than red, though the reverse was found by other workers. Ascorbic acid does not absorb visible light and the effect of such light on the vitamin C of milk is doubtless a part of the general oxidation changes induced in milk by photo-activation. The work of Hopkins(112) very strongly suggests that the lactoflavin of milk acts as a sensitizer. The effect of light on the vitamin C of milk has been confirmed and studied by several other observers(101,102,103,113,114,115).

Unless light is excluded the vitamin C content of milk decreases on keeping, at a rate which depends on the intensity of light. Sharp(116), however, ascribes, the oxidation of vitamin C in milk to a specific enzyme, ascorbic acid oxidase. The existence of this oxidase in milk is denied by Tauber(117) and by Reedman(118). If it were in fact present one would expect it to act much more rapidly and quantitatively.

Arakawa's peroxidase reaction in milk has been found to be connected with the state of the ascorbic acid in milk, no reaction taking place in the absence of the oxidized form(119).

Kon & Watson(104) found that the vitamin C content of colostrum was only slightly higher than that of later milk, but Rasmussen *et al.*(95) reported more marked differences. Garrett *et al.*(56) found a definite relationship between vitamin C and the yellow colour in the milk of individual cows. The vitamin C content of "dairy orange beverages" has been studied by Mack *et al.*(120).

(v) *Vitamin D.* A useful review of the literature on vitamin D in milk has been written by Olson & Wallis(121). The variation of the factor in milk according to season has been studied by Bechtel & Hoppert(122) in the United States, and Morgan and Pritchard(64) in this country. The results are in good agreement, the former authors obtaining a range from 0·11 (February) to 0·9 I.U. per g. of fat (July, August) and the latter 0·07 for January to 0·8 in June. They are also in agreement with the findings of Kon(5), pp. 81, 82) and of Campion *et al.*(123). In dealing with butters of low potency Bechtel & Hoppert at first attempted to concentrate their antirachitic potency by saponification. They found however (in agreement with Kon & Booth(124,125,126)) that the recovery was only partial. They reported success with alcoholic extraction which failed in the hands of Kon and Booth. Wallis(127) has since confirmed the opinion of the latter authors. The recent work of McDougall(128) supports the views expressed in papers(124,125,126).

Coward(28) found an average vitamin D content of 0·76 I.U. per g. for the butters (mostly Empire) for which vitamin A values have already been given on p. 244. The

Danish butters had an average value of only 0·09 I.U. per g. In a previous review(129) the opinion was expressed that direct insolation of the cow may play a dominant role in the formation of the antirachitic substance in milk under normal conditions. This has been confirmed experimentally by Campion *et al.*(123), who have shown that the exposure of the cow to sun and skyshine contributes all, and the pasture none, of the increase in vitamin D potency which takes place in summer. A small amount is supplied by hay in winter. The finding of Krauss and Washburn(13) that cows receiving alfalfa hay produced butter more potent in vitamin D than cows receiving alfalfa A.I.V. silage from the same field points in the same direction. Wallis & Olson(130) have compared alfalfa hay and prairie hay as sources of vitamin D for the cow and found the former to be slightly better, though the butters produced on both treatments had a very low antirachitic potency.

Kon & Henry(131) have found that under similar conditions of feeding and management the butters produced by Shorthorn and Guernsey cows were of equal antirachitic potency. According to Bechtel & Hoppert(122) the same is true of Holsteins and Guernseys. Other things being equal Guernsey milk should therefore be richer in vitamin D than either Holstein or Shorthorn milk.

The fat of colostrum is richer in vitamin D than that from later milk(132), but the difference is relatively smaller than in the case of carotene and of vitamin A.

Working with rats Supplee *et al.*(133) have found that the effectiveness of a given dose of "pure vitamin D" (probably calciferol) varied according to the vector, milk and lactalbumin suspension enhancing the potency.

(*c*) *Other factors.*

(i) *Lactose.* The effect of lactose on growth and on the assimilation of calcium and phosphorus has been studied by several authors(134,135,136). In all cases lactose increased the assimilation of the minerals. Growth was definitely retarded by higher (60–70%) levels of the sugar(135), but not by lower(136). The poor growth performance of animals receiving a large proportion of lactose in their diet is most probably linked with its incomplete assimilation at such levels. One of the signs of this faulty assimilation is an increase in the blood and urinary level of sugar(137,138,139) due to galactose. Galactose is doubtless the causative agent in lactose cataract, but the calcifying action of lactose is a property of the disaccharide itself, as galactose displays no such action at levels at which lactose is active(136). The phenomenon is probably connected with the specific effect of lactose on the *p*H of the contents of the intestinal tract. This effect on the intestine and its bacterial flora is also probably connected with the observation of Morgan *et al.*(140) of the endogenous synthesis of certain components of the vitamin B_2 complex by lactose fed rats. In this connexion Whitnah & Bogart(140a) obtained satisfactory reproduction in rats on synthetic diets containing lactose, but not when they contained sucrose.

It was suggested in the previous review(6) that in its effect on the eye, lactose, as present in whole milk, must be less toxic than the isolated substance. According to present evidence the difference lies not in the lactose but in the protective action of butter fat(141,142). Lactose present in fresh skim milk produces lens changes at least as readily as commercial lactose incorporated in a synthetic ration(142). Schantz *et al.*(138) found that rats on an exclusive diet of milk supplemented with minerals utilized the lactose completely, but on similar skim milk diets there was a leakage of galactose into the urine. When 4% of fat was added to the milk this leakage ceased.

Striking differences in susceptibility to lactose and galactose cataract of various strains of rats have been observed by Mitchell(143), but strangely enough their blood sugar values on a 35% galactose diet were very similar. The degree of galactosemia cannot therefore be solely responsible for the development of the eye opacities.

Bellows(144,145,146) has studied the biochemistry of galactose cataract and has concluded that there is a loss of glutathione from the crystalline lens and that cystine delays the onset of cataract. This latter finding was not fully confirmed by Mitchell & Cook(147) who studied the effects of a variety of agents on galactose cataract and found that the most significant acceleration is obtained by lowering the protein component of the diet from 15 to 5%.

(ii) *Soft-curd milk.* Good articles on the value of soft-curd milk with extensive bibliography will be found in(148,149).

(iii) *Minerals.* Krauss & Washburn(150) studied over a period of one year the iron and copper content and the anaemia-producing properties of mixed Jersey and Shorthorn milk, and found that neither varied appreciably with the season. The iron and copper intake of cows during stall-feeding was about three times that on pasture. Broek & Wolff(151) have measured the Cu, Zu and Mn content of cow's milk in Holland. They also confirm the finding that neither food nor season influences the Cu content of milk. It is suggested however that the level of Mn is affected to a greater extent by feeding, especially when taken in organic form. Orten & Smith(152) have reviewed the rather conflicting literature on goat's milk anaemia and, on the basis of their own experiments, have come to the conclusion that it is not different in type from cow's milk anaemia. Wolf & Tschesche(153) have made quite similar observations which confirm the earlier findings of Kohler *et al.*(154).

Sex differences in susceptibility to milk anaemia have been described(155,156) but the finding that females are more resistant is probably due to difference in growth rate between males and females(157). Beynon(158) expresses the view that copper is not essential to haemoglobin formation and that normal haemoglobin levels can be achieved in rats kept on laboratory evaporated milk containing very little copper and supplemented only with pure iron. Her experimental evidence does not appear to be strong enough to upset the existing view. Sugiura & Benedict(159) have observed the growth of experimental tumours in rats receiving unsupplemented cow's milk, but found no relationship between nutritional anaemia and tumour growth.

Henry & Kon(189) compared on rats the availability of calcium and phosphorus from raw liquid milk and from $CaHPO_4$ and found lower retentions in the latter case.

(iv) *Milk proteins. In vitro*(160) and *in vivo*(161) experiments on the nutritive value of lactalbumin have been carried out by Kik who found that the biological value of this protein measured by the method of Mitchell was higher than that of casein: viz. 84 against 69 at an 8% level, and equal to that of casein supplemented with cystine. Other biological tests also demonstrated the superiority of lactalbumin. *In vitro*, however, lactalbumin was less readily digested by trypsin and erepsin than casein and this work, as well as that of Glassmann & Gologorskaja(162), shows the futility in their present form of *in vitro* digestion experiments.

Rats receiving a diet containing 17% casein were more resistant to infection with *Bact. enteritidis* than rats receiving the same level of protein in the form of wheat gluten or soya bean flour(163).

Harris & Bunker(164) report that diets in which the protein is in the form of casein will not produce rickets in rats even when the Ca/P ratio is 9 : 1 and the phosphorus content of the diet less than 0·2%.

B. *Treated milk*

(*a*) *Heated milk.*

(i) *General.* The widespread recent interest in the problem of the heat treatment of milk, especially in this country, has brought forth a large number of papers dealing with the subject.

Mattick & Golding(165,166) and Wilson & Maier(167) and Wilson(168) have continued the work described in a previous review(129) and Channon & Channon(15) have reported experiments using the same technique as the above-mentioned authors. In all these investigations a diet of raw or heated milk from the same bulk was either supplemented exclusively with a biscuit made from white flour and water(165,166) or contained in addition minerals(167,168,15) or minerals and yeastrel, an extract of yeast(167,168). Wilson used mice in his experiments, the other authors used rats. The results are rather conflicting. Mattick & Golding found that reproduction and lactation were satisfactory on the raw milk, that reproduction was, if anything better, but that lactation was impaired on the same milk after laboratory holder pasteurization and that both failed completely on laboratory sterilized milk. Growth on the sterilized milk was inferior to that on raw milk but pasteurized milk produced growth which on the whole was little, if any, inferior to that on raw milk. Channon & Channon(15) used two types of commercially sterilized milk. They found that their animals developed nutritional anaemia unless minerals were added. On raw milk growth and reproduction were slightly but consistently better, but lactation failed completely on the raw and sterilized milks. Subsequently these workers obtained good lactation on raw milk diets(16). In Wilson's experiments on mice in which commercial holder pasteurized milk was used(167) the outcome depended on the way in which the milk was fed: as far as growth was concerned feeding in open dishes favoured the pasteurized milk group but, when all were fed from inverted tubes, the raw milk mice fared better. The breeding performance was not observed in the "open dish" experiments. In the "inverted tube" technique which favoured raw milk there was little difference in the numbers of litters produced and reared, but the weanlings were heavier on the raw milk.

The difference in performance according to the method of feeding between the two groups of mice was possibly due to better separation of fat from raw milk: when drinking from inverted tubes, mice on raw milk took a product of lower caloric value. They, therefore, probably drank more and obtained more of the essential factors. Satisfactory records of consumption would have settled the point.

No differences in resistance to inoculation or feeding with *Bact. typhi-murium* were found between the raw and pasteurized milk groups(168).

Simonnet *et al.*(169) fed groups of rats, each group consisting of about twelve couples, on an exclusive diet of milk either raw, or pasteurized commercially at 80 and at 90° C. for 36 sec. The rats probably derived some nutritional benefit from their bedding, which consisted of sawdust, straw and filter paper. There was no difference in growth, but reproduction and rearing of young was better on the raw milk.

Krauss(170,171) has briefly reported on the behaviour of rats receiving an exclusive diet of mineralized milk either raw or pasteurized. No difference in growth was noticed.

A claim to the discovery in milk of a new thermolabile growth factor distinct from all known vitamins is made by Arthus(172). The author does not appear to realize that the proteins of milk and vitamin B_1 must have been seriously affected by the very prolonged heating (up to 12 hr. at 100° C.) or autoclaving, to which he exposed the milk. His conclusions are as unorthodox as the name—thérone—given to the hypothetical substance, derived from the first four letters of the name of the person to whom the alleged discovery is dedicated.

Laboratory experiments on raw and pasteurized milk sponsored by the Milk Marketing Board and mentioned in the previous review(6) have been completed and a comprehensive report has been published(173). The experiments dealt with the effect of heat treatment (commercial holder pasteurization) on the total nutritive value, the availability of the salts and the vitamin content of the milk. The last two are dis-

cussed in §§ (ii) and (iii) of this chapter. No difference whatever was found in the growth promoting properties of the two milks when they were fed as the sole diet in equalized amounts to pairs of litter-mate rats. Iron, copper and manganese salts were added to the milks. Moreover, no difference was found in the appetite displayed by the rats for the two kinds of milk. This showed that *ad libitum* feeding would probably yield results similar to those given by the equalized feeding.

Henry *et al.*(11) carried out elaborate experiments, in which the milks already mentioned on p. 243 were compared by paired feeding before and after laboratory holder pasteurization. No difference was found between raw and pasteurized milk whether it was summer or "winter" or whether it was fed as an exclusive diet supplemented only with minerals or as an addition to a basal diet.

Experiments similar to those carried out on the commercially pasteurized milk(173) were extended by Kon *et al.*(174,175,176,177,178,179,180,181) to commercially sterilized milk. With regard to experiments involving the exclusive feeding of milk the authors concluded, as already pointed out in(173), that the level of milk intake is too high to disclose differences even after heat treatment as drastic as sterilization. When the intake of milk was cut down by the addition of cane sugar or of a basal diet, rats on raw milk grew definitely better than those on sterilized, the difference was most probably due to a partial destruction of vitamin B_1 in the sterilized milk. This is further discussed in § (ii) of this chapter.

Cow's milk is the natural food of the calf and experiments on the effect of heat treatment of milk carried out on this animal have therefore a special value. Three such experiments, two dealing with the total nutritive effect of raw and pasteurized milk(182,183) and one with the effect of heating (holder pasteurization) on the availability of the proteins and salts of milk(184), have been published within the time covered by this review. A fourth experiment is not yet available for publication. The outcome of both experiments dealing with the effect of milk on the growth and performance of the calves was similar in that no difference was found between the groups on raw and on pasteurized milk. In one of them(182) milk from tuberculin tested cows was used. In the other(183), in which commercial pasteurized milk was fed, the milk before pasteurization contained viable tubercle bacilli and caused a heavy tuberculous infection of the raw milk calves. It was shown that the infection did not appreciably affect the growth rate. This was in any case very slow and it is conceivable that, given more rapid growth, the results of the experiment would have been different.

(ii) *Vitamins.* Kon and his colleagues studied the fate of various vitamins in commercially pasteurized(173) and in commercially sterilized milk(177,178,179). Lovibond tintometer and spectrophotometric measurements showed that neither carotene nor vitamin A were affected by pasteurization. It was found in animal experiments that pasteurization caused a slight loss in the vitamin B complex. Subsequent fluorometric estimations showed a destruction of about 20% of the vitamin B_1(185). Of the vitamin C originally present in the milk some 20% was destroyed by pasteurization(173). However, the heat treatment cannot alone be blamed for this loss as Kon & Watson(109) have shown that only the reversibly oxidized form of ascorbic acid is destroyed in the process. The reduced ascorbic acid which alone is present in normal milk before exposure to light (p. 247) withstands pasteurization without loss. These findings have since been confirmed by Holmberg(115).

When sterilized milk was studied, vitamin A and carotene were again estimated by two methods. The effect of the heat treatment was negligible, neither method demonstrating a loss in vitamin A. For carotene only, the colorimetric method indicated a loss of about 2%(177). In the same paper the extraction of fat from homogenized sterilized milk is described. The destruction was much more serious in

the case of vitamin B_1. Biological tests showed a loss of some 30% (178). According to later estimations by the fluorometric method the loss amounted to 45% (185). Biological estimation of the vitamin B_2 complex, which probably measured lactoflavin, disclosed no loss of the factor (178). Physical measurements also failed to detect a loss (185). About half of the vitamin C present in milk before sterilization was destroyed by the heat treatment and in view of the severity of the treatment, it is surprising that the loss was not greater, but as the sterilization was largely anaerobic this finding may perhaps be accounted for.

Holmberg (186) studied, by physical and chemical methods, the effect of laboratory "pasteurization" (heating for 30 min. to 70° C. in an open vessel on an open flame and with constant stirring) on vitamin A and carotene of milk and found no destruction. Addition of copper did not influence the results.

Whitnah *et al.* (92) studied the effect of various methods of pasteurization and of the addition of metals on the loss of vitamin C in heat treatment. The loss was always greater in holder than in high temperature short time pasteurization. On keeping, pasteurized samples lost more than raw milk unless pasteurization was carried out in glass or by the flash method in stainless steel. This acceleration was attributed to contamination with copper. Copper was the most efficient catalyst and iron was only 1/20th and chromium and nickel 1/200th as active.

The authors also added copper (0·3 g. daily) to the ration of cows and made the interesting observation that while this addition had no effect on the vitamin C content of the milk, the loss on storage after pasteurization was greater in such milk than in milk from untreated cows. The amount of copper which passed into the milk must nevertheless have been extremely small. According to a brief note by Pratt (187) 9 p.p.m. of manganese cause an increased destruction (in the course of pasteurization) of vitamins B_1 and C but not of vitamin A. Sharp (116) reports that the vitamin C of flash pasteurized milk is more stable on keeping than that of raw milk. This view is not supported by the work of Whitnah *et al.* (92).

(iii) *Other factors.* Recent work shows that commercial pasteurization by the holder method has no adverse effect on the availabilities of calcium, phosphorus and nitrogen of milk, whether these are measured on calves (184) or on rats (173) but that commercial sterilization definitely lowers the biological value of the milk proteins by about 6% (175).

In a study, on rats, of calcium shortage in the "poorer class" diet Coward *et al.* (188) have reached the conclusion that the calcium of pasteurized milk is no more available than the calcium of inorganic salts. It does not seem that this conclusion is fully justified by the experimental data as not total calcium retentions were measured but only ash percentages in femora and humeri and the gain in weight of the milk rats was much greater than that of the rats receiving calcium salts.

The work of Henry & Kon (189) discussed on p. 249 also supports a different view.

The general impression conveyed by the publications on the heat treatment of milk now under review is that properly controlled commercial pasteurization has only a negligible effect on its nutritive value, but that commercial sterilization is, as would be expected, more harmful. Nevertheless, even sterilized milk remains a foodstuff of outstanding merits.

(*b*) *Irradiated and vitaminized milk.*

The excellent review on vitamin D milks by Krauss and Bethke has been brought up to date (190). In addition to a description of the various types of milk fortified with vitamin D which are available in the United States it also gives figures illustrating the increased use of such milks in that country. The popularity of vitamin D milks in the United States is especially interesting as the product does not seem to be making

much headway elsewhere. Further authoritative information will be found in a report of the Council on Foods of the American Medical Association (191).

Laboratory methods for the determination of the antirachitic potency of vitamin D milks are described in (192,193,194). Results of routine tests on such milks sold in New York City are given in (195).

Problems connected with the irradiation of milk with ultra-violet light have been further studied by several authors (196,197,198,199). An investigation of the relative efficiency of various wave-lengths in activating milk and in producing a flavour (196) and a study of the relation of energy input to the stability of the vitamin D generated in milk (198) are of special interest.

Factors in milk affecting its capacity for activation have been studied by Light *et al.* (200) and by Krauss *et al.* (201). The former found that ergosterol, when fed, is not secreted in the milk: 120 mg. of pure ergosterol or 4·3 ounces of yeast daily failed to increase the "activatability" of the milk. This observation is in good agreement with the earlier work of Schönheimer (202) that ergosterol is not absorbed by animals and the recent finding that 7-dehydrocholesterol and not ergosterol is the natural provitamin D (203). Krauss and his colleagues studied the effect of the feeding and breed of the cow, of the season of the year, and of the fat percentage of the milk on its "activatability" by ultra-violet rays and found that the fat percentage and the original activity of the milk are the most important factors. Light *et al.* (200) found that 325,000 I.U. of vitamin D, fed daily to cows as irradiated yeast, produced more potent milk than the same number of units given in the form of irradiated ergosterol. Earlier work on this subject, with which this finding is in partial agreement, has been discussed in the previous review (6).

C. *Milk products*

(*a*) *Cheese.*

Very few papers of interest have come to the reviewer's notice. Day & Darby (204) have studied, by a biological method, the lactoflavin content of several varieties of cheese. American cheese and whey contain only negligible quantities of the black tongue preventing factor (205).

(*b*) *Dried and evaporated milk.*

The lactoflavin content of dried whole milk expressed in Bourquin-Sherman units is given by Levine & Remington (206).

In biological experiments Cannon & Hixson (207) measured the vitamin A content of irradiated and non-irradiated evaporated milk and came to the conclusion that irradiation did not destroy any vitamin. The milks were however purchased on the open market and hence presumably from different sources. Wilkie (208) describes the extraction and saponification of fat from evaporated milk for the estimation of vitamin A.

The biological value of the proteins of dried skimmed milk has been measured by Swaminathan (209,210) by the growth method and by the method of Mitchell. Using adult rats the values for the latter were 89 and 83 respectively at a 5 and a 10% level of protein intake.

Reid (211) gives values for calcium, phosphorus and nitrogen retentions for whole milk powder. Keil (212) fed rats on an exclusive diet of whole dried milk supplemented with iron copper and manganese and found that it did not support reproduction and lactation which became normal on addition of sodium bromide to the diet. In studying the influence of the diet on resistance to infection Watson (213) found that mice, bred and reared on a certain diet containing dried separated milk, were more resistant

to infection with *Bact. typhi-murium* than mice bred and reared on a similar diet from which the milk and certain other components were omitted and the amount of oatmeal increased. She suggested that the increased resistance was due to the milk. On the other hand Church *et al.* (214) reported that mice produced and reared on a diet containing 5% dried whole milk were more resistant to *Bact. enteritidis* infection than mice similarly produced and bred on a different diet containing 33% of the milk, when both groups were, after inoculation, placed on the same purified diet.

II. Nutritional value of milk for human beings

A. *General effects*

The universal interest in nutritional matters and the application of the newer knowledge of nutrition to practical problems is reflected in a large number of publications ranging from voluminous reports of League of Nations Committees to an article in *Punch* (215). As before milk claims a great deal of attention. In addition to articles devoted specially to it, it occupies a prominent place in most of the official pronouncements on nutritional problems and policy.

A revised and amplified version of the *Report on the Physiological Bases of Nutrition* has been published (216). An exhaustive survey of the milk problem written by distinguished experts has also appeared (217). It is rather disconcerting to find that these two publications of the Health Committee of the League of Nations are not in agreement on certain points, thus the former considers milk, whole or skimmed, to be a good source of vitamin B_1, whereas the latter finds it of little account in this respect. This difference in opinion is the more surprising as the values quoted in (216) for the vitamin B_1 content of milk, 50–57 I.U. per l., are almost certainly too low (cf. p. 246). The view, expressed in the same publication, that milk although itself poor in iron, renders more effective the iron contained in the diet, would probably not be unanimously accepted by students of nutrition. Further important publications of the League of Nations deal with the food value of milk, its place in human nutrition, its role in improving existing dietaries, give data concerning its consumption in various countries and make suggestions as to future research on milk (218,219,220,221). In this country the first report of the Advisory Committee on Nutrition of the Ministry of Health has been published (222). Much space is devoted to milk and milk products. A memorandum of the Children's Minimum Council (223) should be consulted. An excellent pamphlet on correct nutrition has been written in simple language by Bigwood (224). The quantities of milk recommended by him are smaller than those given in (216). A bulletin of the Imperial Bureau of Animal Nutrition (225) summarizes recent nutritional investigations throughout the British Colonial Empire. Another dietary survey of interest is that of Schenk (226) dealing with the diet of competitors at the last Olympic Games. In both, interesting data on milk will be found. The same applies to the well-known survey by Orr (227).

Less than half of the possible recipients of cheap and of free milk under the Milk-in-Schools Scheme were taking the milk in October 1935. The extensive enquiry of Wright (228) and those of Murray (229) and of Williams (230) have done much to explain the reasons for this abstention.

Turning now to the results of numerous investigations of the value of milk in supplementing existing diets we find, as would be expected, that the poorer the original diet the more obvious is the beneficial effect of such an addition. Thus Aykroyd & Krishnan (231) and Krishnan & Mitra (232) obtained statistically significant improvements in height and weight and marked improvements in general condition in relatively small (20–60) groups of children receiving the equivalent of 1 oz. of dry

skim milk daily, when compared with similar groups of control children receiving a supplement of millet or of ordinary biscuit of equal caloric value. The basal diet in both cases was a typical South Indian one. The experiments were of short duration (10–14 weeks) and this, together with the smallness of the groups yielding statistically significant results, emphasizes the deficiencies of the original diet.

Equally striking results have been reported from Malaya(233) but the data have not been statistically examined. In England an interesting experiment on specially selected school children was carried out at Norwich(234). The children were chosen "on physical grounds—chiefly anaemia, malnutrition and debility". Roughly half of them were given one pint of milk daily at school. No caloric allowance was apparently made for the controls. After 15 months both girls and boys in the milk group were heavier and taller than the corresponding controls. The groups consisted of 90–110 children. No statistical examination of the results is reported.

Experiments on pre-school negro boys are reported from the United States(235) but the information given in this preliminary report is scanty. An enquiry into the diet of Hindoo and Moslem children in an East African School(236) speaks in favour of milk drinkers.

In efforts to reduce the high maternal mortality rates in the distressed areas the National Birthday Trust Fund tried various schemes(237,238,239). Of these, additional medical and nursing facilities alone were without effect, but the distribution of food alone or combined with the medical facilities reduced the puerperal death rate to 1·64% while groups not receiving the additional food had a death rate of 6·39%. The additional food included fresh and dried milk and ovaltine.

The dietary conditions prevailing in the distressed areas are illustrated by a survey by Harry of the consumption of milkstuffs and meatstuffs in the Rhondda Valley(240).

The beneficial effect of an addition of milk proteins (plasmon) to an institutional diet is illustrated by Hoske & Paul(241).

The problem of the nutritive value of milk for human beings has been approached from a new angle by Drummond *et al.*(242) who gave simultaneously to groups of children in two institutions near London normal winter milk and milk produced by cows from the same herd receiving artificially dried grass. The dried grass, when fed, replaced a part of the hay and roots of the winter ration and one would expect such milk to be richer in vitamin A and carotene and, if anything, poorer in vitamin D, than the winter milk. No differences were found between the effects of the two milks, possibly because the basal diets may have contained an adequate supply of vitamin A. Also the carotene content of the artificially dried grass was low and the difference between the milks was not sufficiently marked. It is to be hoped that this interesting experiment will be repeated under more favourable conditions.

B. *Effects of treatment*

(*a*) *Heated milk.*

There is very little to record under this heading. The preliminary report on the large scale experiment carried out in schools in England and Scotland is not yet officially available.

Graham & Morris(243) carried out balance experiments on two children, aged 7 and 10 years, on the retention of Ca, P and N when about 50% of the caloric value and 70% of the protein were supplied either by raw or by boiled milk from the same source. In several trials no difference was found between the two milks. The authors wisely point out that short one-week periods may lead to fallacious results.

Blackham(244) has written an interesting article on the use of condensed milk in warm climates.

(*b*) *Irradiated and "vitaminized" milk.*

It was pointed out in the previous review(6) that the vexing problem of the relative efficiency for the human infant of vitamin D from different sources was becoming clearer as a result of more careful clinical work.

In the two years which have since elapsed, further marked progress has been made and it is gratifying that the solution, in any case as it now appears, is simpler than one would have expected several years ago. It now seems that for the human infant, the relative potency of vitamin D from fish liver oils, irradiated ergosterol, irradiated cholesterol and from the various types of vitamin D milk is the same as for the rat. Rat-unit for rat-unit all these sources are now believed to be equally efficient. In other words the human being and the rat do not seem to differentiate between vitamin D_2 (calciferol, irradiated ergosterol) and vitamin D_3 (the vitamin—or vitamins of fish liver oils, irradiated 7-dehydrocholesterol). In this they differ from the chicken.

Several excellent reviews have recently appeared dealing with the subject in an exhaustive fashion(245,246,247) and the reader is referred to them for a bibliography and a full discussion of the evidence which has led to the conclusions briefly summarized above. A summary by Drake(248) of the investigations carried out in Toronto on over 1200 children, should also be seen as well as a very good short review on vitamin D milks by Kramer(249). The reviewer is very grateful to Dr Jeans who allowed him to see a copy of the article(247) before publication. In view of the latest conclusions one might legitimately ask why such definite differences between various antirachitic agents were reported in the earlier studies which are discussed in(6) and(129). It will be recalled that at the time vitamin D milks were considered by several observers to be superior to cod-liver oil and both products were believed to exceed the efficiency of irradiated ergosterol. In the first instance ease of administration, especially in out-patient studies may play a definite role. In this respect vitamin D milks score over more concentrated sources and it is possible that this accounts for the slight difference in favour of fortified milks observed in their careful studies by Eliot *et al.*(250). Another factor of greater importance is the state of concentration or dispersion of vitamin D. Laboratory experiments to this effect(133) have been described on p. 248. Their clinical counterpart will be found, in addition to the earlier work mentioned in the previous review(6), in the more recent papers of Lewis(251), Erben(252) and Shelling(253). These show that irradiated ergosterol in a dispersed state is much more effective than in a high concentration.

Very low levels of vitamin D have been found effective in preventing or arresting the development of rickets(6) but such levels may be insufficient for optimal growth or optimal deposition of minerals. An intake of at least some 300–400 I.U. daily is probably necessary for this purpose(247). The various fortified milks commercially available in the United States contain from 135 to 430 I.U. per American quart(190) and may therefore have to be supplemented with more potent sources of vitamin D. The high prices of such milks are discussed in(254).

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